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Evolutionary relationships in Afro-Malagasy Schefflera (Araliaceae) based on nuclear and plastid markers

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EVOLUTIONARY RELATIONSHIPS IN AFRO-MALAGASY *SCHEFFLERA* (ARALIACEAE) BASED ON NUCLEAR AND PLASTID MARKERS

A thesis submitted in partial fulfillment of the requirements for the degree of M.S. Biology at Virginia Commonwealth University.

by

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Table of Contents

Acknowledgements ii

List of Tables v

List of Figures vi

Abstract 1

Introduction 3

Materials and Methods 10

Results 14

Discussion 18

Literature Cited 28

Tables 34

Figures 40

Vita 66

List of Tables

Table 1: Species used for DNA samples used in this study, including geographic range and voucher information 34

Table 2: Oligonucleotide primers used for PCR amplification and DNA sequencing 39

List of Figures

Figure Legends: 40

Figure 1: The strict consensus of 51,600 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the nuclear ETS rDNA spacer. Tree length = 279 steps, CI = 0.675, RI = 0.954. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 45

Figure 2: The strict consensus of 96,600 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the nuclear ITS rDNA spacer. Tree length = 271 steps, CI = 0.708, RI = 0.959. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 46

Figure 3: The strict consensus of 100,000 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Tree length = 565 steps, CI = 0.665, and RI = 0.951. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 47

Figure 4: The strict consensus of 87,700 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Tree length = 655 steps, CI = 0.612, RI = 0.918. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are

provided above the branches; bootstrap values less than 50% are recorded as "<". Gray arrows indicate specimens whose placement has moved considerably from other analyses 48

Figure 5: The strict consensus of 94,200 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Tree length = 1,238 steps, CI = 0.608, RI = 0.929. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 49

Figure 6: The best tree (log likelihood = –2,279.056) based on maximum likelihood (ML) analysis of 123 sequences from the nuclear ETS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 50

Figure 7: The best tree (log likelihood = –2,612.7117) based on maximum likelihood (ML) analysis of 123 sequences from the nuclear ITS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 51

Figure 8: The best tree (log likelihood = –5,075.1868) based on maximum likelihood (ML) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 52

Figure 9: The best tree (log likelihood = –8,530.1115) based on maximum likelihood (ML) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-*

rpl32. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<". Gray arrows indicate specimens whose placement has moved considerably from other analyses 53

Figure 10: The best tree (log likelihood = –14,328.1040) based on maximum likelihood (ML) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<" 54

Figure 11: The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the nuclear ETS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the Branches 55

Figure 12: The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the nuclear ITS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the Branches 56

Figure 13: The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches 57

Figure 14: The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the

right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches. Gray arrows indicate specimens whose placement has moved considerably from other analyses 58

Figure 15: The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches 59

Figure 16: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with geographic locality mapped onto branches using MacClade (version 4.08). DIVA (version 1.1a) output is provided above branches to indicate ancestry for divergence nodes. "A" corresponds to continental Africa and "B" corresponds to Madagascar 60

Figure 17: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with carpel number states mapped onto branches 61

Figure 18: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with leaf composition character states mapped onto branches 62

Figure 19: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with inflorescence arrangement states mapped onto branches 63

Figure 20: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with pedicel lengths mapped onto branches..... 64

Figure 21: The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with bract persistence mapped onto branches..... 65

Abstract

EVOLUTIONARY RELATIONSHIPS IN AFRO-MALAGASY *SCHEFFLERA* (ARALIACEAE) BASED ON NUCLEAR AND PLASTID MARKERS

By Morgan Robert Gostel, M.S. Biology

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The genus *Schefflera* is the largest in Araliaceae, with approximately 900 species. Recent studies have shown that *Schefflera* is polyphyletic and represents no fewer than five distinct clades, each corresponding to a specific geographic region including Asia, continental Africa and Madagascar, Melanesia, the Neotropics, and a small clade distributed throughout several islands in the insular Pacific Ocean. The Afro-Malagasy clade contains nearly 50 species distributed throughout tropical, sub-Saharan Africa, Madagascar, the Comoros, and the Seychelles islands. Previous studies have suggested that this group is monophyletic, identifying two smaller subclades within Afro-Malagasy *Schefflera* corresponding roughly to informal groups identified as “Meiopanax” and “Sciodaphyllum” on the basis of morphology. Using sequence data from nuclear rDNA spacers and plastid markers derived from 32 of the 48 currently circumscribed species of Afro-Malagasy *Schefflera*, this study tested the monophyly of Afro-Malagasy *Schefflera* and of each of its two proposed subclades. Trees based on this molecular data were used to examine patterns of morphological evolution and biogeography among species in the clade. Results support the monophyly of Afro-Malagasy *Schefflera* and both subclades, which

correspond closely to “Meiopanax” and “Sciodaphyllum” which are herein referred to as *Neocussonia* and *Astropanax*, respectively. Additional interspecific relationships were examined, which provides evidence for hybridization among several species. *Schefflera myriantha*, the most widely distributed species of Afro-Malagasy *Schefflera*, is paraphyletic with respect to two other species, *S. humblotiana* and *S. monophylla*. Many morphological features historically used to distinguish species of Afro-Malagasy *Schefflera* appear to be evolutionarily labile, with a history of gains and losses (e.g., reduction in leaflet number, which occurs independently in both subclades). Biogeographic analyses suggest an African ancestry for the entire Afro-Malagasy *Schefflera* clade, and for both subclades, with two independent divergence events to Madagascar.

INTRODUCTION

Taxonomic History. The genus *Schefflera* represents the most speciose of the 41 genera currently recognized in the angiosperm family Araliaceae (Frodin and Govaerts 2003). Current estimates of species diversity in Araliaceae indicate greater than 1,600 species, with *Schefflera* comprising at least half of those (Plunkett et al. 2005). The taxonomic history of *Schefflera* dates back to the type collection of *S. digitata*, an endemic of New Zealand, from Forster & Forster (1775). Since its original description, *Schefflera* has been subject to a turbulent taxonomic history. The generic definition has been modified several times, primarily to place emphasis on different morphological features and often to include (or "lump") previously distinct genera that share morphological characteristics. The early efforts to broaden the circumscription of the genus were undertaken by Baillon (1878, 1879), Harms (1894-1897) and Viguier (1906, 1909). More recently, similar approaches have been undertaken by Frodin (1975), who greatly expanded the genus to include all woody araliads having palmately compound leaves, ligulate stipules, and valvate petal aestivation, while lacking armaments and articulated pedicels (Frodin 1975). Since its implementation, this most recent definition of *Schefflera* has resulted in explosive growth of the genus, due in large part to inclusion of segregate genera and supplemented by focused studies in the field and herbaria.

Molecular phylogenetic studies of *Schefflera* have advanced the understanding of this complex genus. Recent, phylogenetic analyses of Araliaceae and, more specifically, *Schefflera* have uncovered extensive polyphyly in the genus (Plunkett et al. 2004, 2005). Investigations by Plunkett et al. (2005) identified five large, distinct clades of *Schefflera*, distributed across the phylogenetic tree of Araliaceae. These five clades correspond strongly to particular geographic areas, and have been informally named Asian *Schefflera*, Neotropical *Schefflera*, Melanesian (or

Pacific) *Schefflera*, Afro-Malagasy *Schefflera*, and *Schefflera* sensu stricto (Plunkett et al. 2005). Reconstruction of these relationships was based on a representative but somewhat limited sampling of species from throughout the genus designed to test the monophyly of *Schefflera*, to identify subclades, and, once identified, to initiate more extensive studies of relationships within each clade. To that end, several research projects focused on recircumscription of these clades are currently under study (e.g., Melanesian and Neotropical *Schefflera*). The goal of the present study is to clarify relationships within the Afro-Malagasy *Schefflera* clade.

Evidence from the most recent studies of *Schefflera* (Plunkett et al. 2005) suggest that the Afro-Malagasy clade is part of a large basal polytomy in Araliaceae. Eight additional small lineages have also been placed in this basal polytomy, and include: *Seemannaralia* + *Cussonia*, *Cheirodendron* + *Raukaua*, *Schefflera* sensu stricto, *Osmoxylon*, *Harmsiopanax*, *Astrotricha*, *Cephalaralia*, and *Motherwellia* (Plunkett et al. 2005), together with three much larger clades (*Aralia-Panax*, the *Polyscias-Pseudopanax* clade, and the Asian Palmate clade). One of the smaller clades, comprising *Cussonia* and the monotypic *Seemannaralia*, is endemic to Africa. At present, a phylogenetic study is underway for *Cussonia*, a genus of approximately 20 species (De Villiers and van Wyk 2006; De Villiers unpublished), but resolution of Afro-Malagasy *Schefflera* relative to other clades in the basal polytomy is beyond the scope of this study, which is focused exclusively on relationships within the clade itself.

The early taxonomic history of Afro-Malagasy *Schefflera* dates to the 19th Century, to Seemann's (1865) study, which initially described African collections in the genera *Astropanax* and *Sciadophyllum*. It was not until the end of the 19th Century that Harms (1894) began placing African araliads in *Schefflera* based on an expanded definition that encompassed the morphological features of several segregate genera. Much later, in the mid-20th Century,

Bernardi and Bamps revisted the African and Malagasy species of this genus. Bernardi's (1969) work focused on endemics from Madagascar and the nearby Comoro islands. His treatment of the genus described three new species as well as two new varieties of *Schefflera* and moved ten other species to *Schefflera* from *Cussonia*. Two species (*S. umbellifera* and *S. bojeri*) had been previously assigned to *Schefflera* by Baillon (1878) and Viguier (1906), respectively. By contrast, Bamps' (1974) study of *Schefflera* focused exclusively on representatives from continental Africa. In Bamps' work, two new species were described, adding to the two already recognized in *Schefflera*. Together, these studies resulted in the recognition of a total of 29 species of Afro-Malagasy *Schefflera*. It is from this foundation that the contemporary systematic studies of Afro-Malagasy *Schefflera* now emerge. The past three decades have brought the number of hypothesized species of Afro-Malagasy *Schefflera* to 48 according to Lowry (pers. comm.).

Presently, Frodin (in Plunkett et al. 2005) places the species of *Schefflera* from Africa and Madagascar within two subgenera on the basis of morphological characteristics, informally named "Meiopanax" and "Sciodaphyllum". By contrast, Bernardi (1969) identified three series of Malagasy *Schefflera*, namely *Racemosae*, *Myrianthae*, and *Anticipes*. The nine representatives sampled in Plunkett et al. (2005) provided evidence for two subgroups, but this sampling was too limited to draw more detailed conclusions. The first clade corresponds closely with Frodin's "Meiopanax" group, which is here referred to as *Neocussonia* based on a genus recognized by Hutchinson (1967) for Malagasy species formerly placed in *Cussonia* (*Schefflera bojeri*, *S. monophylla*, and *S. myriantha*) and one from Africa (*S. umbellifera*). The second clade corresponds to Frodin's widespread "Sciodaphyllum" group, which he recognized on the basis of a "generalized" or "unspecialized" morphology. However, "Sciodaphyllum" itself is

polyphyletic and the type species belongs to another clade (Neotropical *Schefflera*), therefore species of Afro-Malagasy *Schefflera* belonging to this group are referred to as *Astropanax* based on a generic group established by Seemann (1865). Currently, 32 species are recognized in the *Neocussonia* clade, while 16 have been placed in *Astropanax* (Lowry, pers. comm.).

Geography. The geographic distribution of these species raises questions regarding the origin and diversification of Afro-Malagasy *Schefflera*. Madagascar, the fourth largest island in the world, is the primary center of diversity of Afro-Malagasy *Schefflera*, with 33 of the 48 species. The island is separated from the African continent by the Mozambique Channel and lies approximately 400 km to the east of Mozambique and Tanzania. The Comoros are a small network of islands and islets that lie between Africa and the northern tip of Madagascar, having a single species of *Schefflera* (*S. myriantha*, which is also found in both Madagascar and continental Africa). The Seychelles, another small network of islands located to the north of Madagascar in the Indian Ocean, also has a single species of *Schefflera* (*S. procumbens*). Another 15 species are distributed across the African continent's sub-Saharan tropics. Both subgroups, *Neocussonia* and *Astropanax*, contain species endemic to both the continent and the islands to the east. *Neocussonia*, however, is more diverse in Madagascar, whereas *Astropanax* is better represented in Africa. The geographic origin of these groups, either in Africa or Madagascar, remains a fundamental question in understanding the diversification in this clade.

Originally part of Gondwanaland, present-day Madagascar, India and the Seychelles are estimated to have separated from Africa between 165 and 175 mya (Besse & Courtillot 1988, Schettinot & Scotese 2005, Ali & Aitchison 2008) and tectonic movement between the two landmasses has been shown to have largely stopped approximately 125 mya (Rabinowitz et al.

1983). Later separation of India and what now comprises the Seychelles from Madagascar has been dated to roughly 88 mya (Storey et al. 1995). The ability to test hypotheses regarding biogeographic divergence among related plant groups has been facilitated by an abundance of paleomagnetic and geophysical data and has contributed to a growing interest in phytogeography (Schatz 1996, Yuan et al. 2005). Recent molecular phylogenetic reconstruction has provided evidence for both vicariance and dispersal events in the floras endemic to landmasses once a part of Gondwanaland and this has fueled debate regarding patterns of biogeography (Crisp & Cook 2007, Donoghue & Smith 2004, McGlone 2005). Historically, phylogenetic divergence in many elements of the southern hemisphere flora and fauna has been attributed to Gondwanan vicariance, but more recent studies have challenged these conclusions (Sanmartín & Ronquist 2004). In particular, the application of molecular-dating techniques suggests long distance dispersal or a combination of vicariance and dispersal events is often responsible for much of the diversity once attributed to the appearance of geological barriers (Cook & Crisp 2005, Barker et al. 2007, Weeks et al. 2005, Zerega et al. 2005).

Broader phylogenetic connections between species of *Schefflera* from Africa-Madagascar to those species from Malesia and the Pacific can be largely ruled out based on previous studies, which demonstrate that these taxa belong to three unrelated clades (see Plunkett et al. 2005). A better point of comparison within Araliaceae for the biogeography of Afro-Malagasy *Schefflera* can be found in the study of *Polyscias* by Plunkett et al. (2004), which focused on phylogenetic relationships among the species from the Indian Ocean Basin (IOB). IOB *Polyscias* is distributed across a region that includes continental Africa, Madagascar, the Comoros, and the Mascarene islands. Of particular note is one clade identified as the "*Polyscias fulva* group", representing 10 species endemic to Madagascar (3 spp.), the Comoros (1 sp.) and Africa (6 spp.) (Plunkett &

Lowry 2010, Plunkett et al. 2004). Within the *Polyscias fulva* clade, it was suggested that multiple dispersal events between Africa and Madagascar were likey responsible for its diversity (Plunkett & Lowry 2010); similar hypotheses will be tested in the present study among species of Afro-Malagasy *Schefflera*.

Habit & Morphology. Afro-Malagasy *Schefflera* have been described as trees, shrubs, or lianas, often epiphytic, with some species reaching heights of 30 m (Tennant 1961, Bamps 1974). Historically, Afro-Malagasy *Schefflera* have been identified by a mixture of morphological features including both umbellate and racemose inflorescence arrangement, 2–3, 4–5, or 6–9 carpels, simple or palmately compound leaves, as well as other combinations of leaf and inflorescence characters (Bamps 1979, Bernardi 1980). Many combinations of states for these characters are present in species informally grouped in both *Neocussonia* and *Astropanax*.

Assignment of the morphological character states listed above has, in several cases, failed to reflect reliable species definitions for some taxa in *Astropanax* and *Neocussonia*. Bernardi (1974) noted problems with species delimitations in his treatment of the group, which emphasized leaf shape. In particular, Bernardi noted the great variation in the leaf shape of *Schefflera longipedicellata* not only among distinct specimens, but also in the same individual. Bernardi (1969) also suggested the possibility that hybridization between two Malagasy species, *S. longipedicellata* and *S. monophylla*, may have produced the character states exhibited by *S. staufferana*. A survey of morphological characters among the species of Afro-Malagasy *Schefflera* will help to identify the sources of confusion leading to these ambiguous determinations and may assist in revising species circumscriptions that reflect the full range of variation in each operational taxonomic unit.

Character-state mapping may help to identify morphological features defining subclades and to test whether some character states share a single evolutionary origin (e.g., the reduction of digitately compound to simple leaves) or if these features arose multiple times during the evolutionary history of the clade. A deeper understanding of other character states, even if homoplasious, may prove useful as features for the construction of species-identification keys.

Objectives & Scope. The findings outlined in Plunkett et al. (2005) suggest that the species of *Schefflera* from Africa and Madagascar form a monophyletic group. The current study employs a greatly expanded sampling of the species from this region to test this finding and to explore further species-level phylogenetic relationships. Previous studies have demonstrated the utility of molecular data in phylogenetic reconstruction among the species and genera of Araliaceae, using multiple molecular markers from both the nuclear (ITS and ETS) and plastid (notably, *trnL-trnF*) genomes (e.g., Eibl et al. 2001; Plunkett et al. 2004, 2005; Tronchet et al. 2005; Nicolas & Plunkett 2009). A repeated problem in species-level molecular phylogenetics of plants has been the scarcity of informative plastid markers, resulting in poorly resolved trees at this level (Miller et al. 2009). In response to this deficit, recent studies have identified several plastid sequence regions that accumulate mutations rapidly enough to resolve relationships among taxa at the species level in many groups of angiosperms (Shaw et al. 2005, 2007; Miller et al. 2009). Several of these plastid markers were selected as candidates for this study, but three intergenic spacers were ultimately chosen for sequencing across all sampled taxa, *trnK-rps16*, *ndhF-rpl32*, and *rpl32-trnL*. In addition to these three markers, two nuclear spacers (ITS and ETS) have been identified in previous studies that exhibit sufficient variation to help resolve interspecific relationships in Araliaceae (e.g., studies of *Polyscias* and *Meryta*; see Eibl et al.

2001, Plunkett & Lowry 2010, Tronchet et al. 2005) and these were also used in the present study. Sequences for all five of these markers were derived from a representative sample of 18 of the 48 currently recognized species of Afro-Malagasy *Schefflera*, with a primary objective to test the presumed monophyly of Afro-Malagasy *Schefflera* under the current system of classification, to explore patterns of interspecific relationships, to test biogeographic hypotheses regarding ancestral origins, and to identify morphological features that can be used in diagnosing subgeneric and interspecific groups of taxa.

METHODS & MATERIALS

Sampling. Comprehensive sampling of all 48 species of Afro-Malagasy *Schefflera* was attempted, but the lack of material from 16 species (viz., *Schefflera abyssinica* (Hochst ex. A. Rich) Harms, *S.* "ambrensis" Lowry ined., *S.* "decaryi" Lowry ined., *S.* "gentryi" Lowry ined., *S. evrardii* P. Bamps, *S. hierniana* Harms, *S.* "kalambatrensis" Lowry ined., *S.* "lewisiae" Lowry ined., *S.* "masoalensis", *S.* "perieri" Lowry ined., *S. procumbens* (Hemsl.) F. Friedmann, *S.* "ranomafanensis" Lowry ined., *S.* "sainteucei" Lowry ined., *S. stuhlmannii* Harms, *S. urostachya* (Engl.) Harms, and *S. weibeliana* Bernardi) resulted in an incomplete but still representative sampling. Of the 16 species left unsampled, ten are currently known only from the type specimen. A total of 160 accessions representing the remaining 32 species were included in this study (see Table 1). Most material was available from fresh field collections, dried on silica gel, but 25 of the 160 samples were derived from older herbarium specimens. Many taxa are represented by multiple specimens to test for monophyly and/or the potential for interspecific hybridization. Outgroup taxa were selected on the basis of relationships observed in previous

studies (i.e., Plunkett et al. 2005) and included species of *Cussonia*, *Schefflera* sensu stricto, *Osmoxylon*, *Astrotricha*, and the monotypic *Seemannaralia*.

Extraction, amplification and sequencing. For each accession, total DNA was extracted using the DNeasy Plant extraction kit (QIAGEN Inc.) or a modification of the protocol described by Alexander et al. (2007). Selected DNA regions were amplified using the polymerase chain reaction (PCR) for each accession with a combination of existing and newly developed primers for each spacer (see Table 2 for list of primers). PCR reaction conditions included 0.5 μL of both forward and reverse primers (5 μ*M*), 0.5 μL spermidine (4 m*M*), 2 μL total DNA, and 5 μL of either the Jumpstart REDTaq ReadyMix (SigmaAldrich) or the GoTaq Green Master Mix (Promega Corp.) *Taq* polymerase mixes. With the exception of three modifications for *trnK-rps16* and *ndhF-rpl32*, all thermocycler protocols for PCR amplification included a pre-soak step of 4 min at 94ºC, followed by 35 cycles of 30 sec at 94ºC (denaturation), 1 min at 52ºC (annealing), and 50 sec at 72ºC (extension), and then a single post-soak of 72ºC for 4 min. Due to lower primer melting temperatures, thermocycler protocols were slightly modified for both *ndhF-rpl32* and *trnK-rps16* by lowering the annealing temperature to 48ºC (for *trnK-rps16*) or 50ºC (for *ndhF-rpl32*) and by extending the annealing time to 90 sec (for both markers). In addition, the extension time was modified to 135 sec for *ndhF-rpl32* due to the increased length of this marker. PCR products were purified using 1.5 μL exonuclease I and 3 μL shrimp alkaline phosphatase per 5 μL of product (USB Corp.). Purified PCR products were sequenced directly using a thermocycler program of 20 sec at 94ºC, 15 sec at 55ºC, and 1 min at 60ºC for 30 cycles. Sequencing reactions were carried out using DYEnamic ET Terminators (GE Healthcare, Inc.) or BigDye Terminator (vers. 3.1, Applied Biosystems Corp.) and then purified using the

MultiScreen$_{384}$ SEQ filtration system (Millipore Corp.) or the BigDye XTerminator purification kit (Applied Biosystems Corp.). Capillary gel electrophoresis of cleaned products was performed on a MegaBACE 1000 DNA Sequencing System (GE Healthcare, Inc.) or an ABI 3730 DNA Analyzer (Applied Biosystems Corp.) and then assembled and edited using the Sequencher 4.7 software package (Gene Codes Corp.). Sequence alignment was adjusted manually following an initial alignment using ClustalX (Thompson et al. 1997). Informative indels resulting from sequence alignment were coded as binary (presence/absence) characters according to the method provided by Giribet and Wheeler (1999). All sequences will be deposited in the GenBank database.

Phylogenetic Analyses. Three distinct approaches to phylogenetic analysis were used in this study, including maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Five separate datasets were used, including (1) ITS, (2) ETS, (3) combined ITS + ETS, (4) the plastid markers (treated together), and (5) a set combining sequences from the two nuclear and three plastid markers. To test for congruence among the separate datasets, the incongruence length difference (ILD) test of Farris et al. (1995) was performed using the partition homogeneity test in PAUP* 4.0b10 (Swofford 2002). Three partitions were established, representing the ITS, ETS, and combined plastid markers. An ILD test was performed comparing all three partitions simultaneously, as well as separate, pairwise ILD tests. For model based approaches (ML & BI), jModelTest (Posada 2008) was used to select the most appropriate model of sequence evolution and to maximize computational accuracy.

Maximum parsimony analyses were performed using PAUP* and a two-step protocol modified from Plunkett et al. (2005). In the first step, a heuristic search of 1,000 replicates was

generated by random, stepwise addition and TBR branch swapping, but saving no more than 100 trees per replicate. The strict consensus from this initial search was then loaded as a topological constraint for a second heuristic search that was performed following the same protocol as the first step (1,000 reps, saving 100 trees per replicate) but saving only shortest-length trees that did not agree with the topological constraint. If no additional shortest-length trees were recovered, the strict consensus from the first analysis was used as a conservative estimate of phylogenetic relationships. Bootstrap values were calculated from 1,000 replicates using PAUP*.

The model-based analyses were performed using either GARLI (version 0.96; Zwickl 2006) for ML or MrBayes (version 3.2.1; Huelsenbeck and Ronquist 2001) for BI. ML analyses were performed applying mostly the default parameters in GARLI and MrBayes, namely the GTR + Γ + I model using four variable rate categories. For ML analyses, the maximum number of generations was set to 5,000,000, saving the ML tree with the best score. ML bootstrap values were also calculated using GARLI (with the same parameters). Bayesian inference used model of sequence evolution closest to that indicated by jModelTest as selected by the Akaike information criterion (AIC), with chains run for 10 million generations for the combined nuclear + plastid dataset and 4 million generations for all other datasets. Markov Chain Monte Carlo (MCMC) was implemented using four chains, sampling every 1,000 generations.

Morphological character assessment. Morphological data were collected from specimens deposited at Musée National d'Histoire Naturelle in Paris (P) and the Jardin Botanique National de Belgique (BR). The following five characters were coded as binary states: inflorescence arrangement (0: umbellate, 1: racemose), number of carpels (0: 2–3, 1: 4–5, and 2: ≥ 6), leaf morphology (0: exclusively unifoliolate, 1: 1–3 leaflets, 2: >3 leaflets), pedicel length

(0: absent, 1: 0.1–5mm, 2: ≥ 5mm), and retention of bracts (0: bracts persistent, 1: bracts caducous). Other characters (e.g., bract size and petiole length) were identified as potentially informative, but omitted from formal character analysis due to limited access to herbarium materials. Morphological character states were mapped onto the combined ITS + ETS tree using MacClade version 4.08 (Maddison & Maddison 2005).

DIVA. Biogeographic relationships were explored using DIVA 1.1a (Ronquist 1996, 1997), which assigned ancestral areas to internal nodes on a fully resolved ML phylogeny produced from a reduced sample set (decreasing the number of terminals, but maintaining the basic topology), using two areas of endemism (continental Africa and Madagascar). Presence or absence of taxa in each area of endemism was coded as a binary character.

RESULTS

Sequence characteristics. A total of 160 accessions was sequenced for each of the five DNA spacer regions employed in this study, although four specimens did not amplify for the *ndhF-rpl32* marker and one specimen did not amplify for the *trnK-rps16* marker. After examining the sequences for redundancy, 37 samples were removed because they shared identical sequences with others in the dataset. The remaining 123 accessions were used for phylogenetic analyses. Uncorrected pairwise distances were calculated between all sequences for each molecular marker (treating the three plastid markers separately). Pairwise distances were also calculated for plastid markers using datasets with and without coded gaps.

The length of the ETS sequences ranged from 375 to 449 nucleotides, with a total aligned length of 474 characters (103 of which were parsimony informative). Nucleotide percentages for ETS were A = 17.7%, C = 28.6%, G = 24.5%, and T = 29.1%. Average pairwise distance among

ingroup taxa was 4.1% for ETS sequences, the greatest distance between two ingroup taxa was 9.7% (between *Schefflera goetzenii* 3503 and *S.* "vohimenensis" 2378), while 204 pairwise combinations provided distance values of zero.

The length of the ITS sequences ranged from 594 to 662 nucleotides, with a total aligned length of 675 characters (95 of which were parsimony informative). The nucleotide composition for ITS was A = 21.8%, C = 30.9%, G = 28.8%, and T = 18.5%. Average pairwise distance among ingroup taxa was 2.5% for ITS sequences, the greatest distance between two ingroup taxa was 5.5% (between *Schefflera myriantha* 1498 and *S. vantsilana* 152), while 124 pairwise combinations provided distance values of zero.

Aligned plastid sequences produced fewer parsimony-informative characters relative to the nuclear spacers. The length of the *trnK-rps16* sequences ranged from 818 to 1,099 nucleotides and produced an aligned total of 1,188 nucleotide characters and 6 coded indels (for a total of 36 parsimony-informative characters). Nucleotide percentages for *trnK-rps16* sequences were A = 41.9%, C = 14.9%, G = 14.6%, and T = 28.6%. Average pairwise distance among ingroup taxa was 0.5% for *trnK-rps16* sequences, the greatest distance between two ingroup taxa was 3.8% (between *Schefflera tessmannii* 2107 and *S.* "floretii" 7172), while 1,262 pairwise combinations provided distance values of zero. One specimen (*Schefflera* "floretii" 7160) did not amplify for *trnK-rps16* and this missing data was coded as ambiguous (N).

Length of the *rpl32-trnL* sequences ranged from 664 to 895 nucleotides and an aligned total of 944 nucleotide characters and 4 coded indels (for a total of 47 parsimony-informative characters). Nucleotide percentages for *rpl32-trnL* sequences were A = 35.7%, C = 14.7%, G = 12.5%, and T = 37%. Average pairwise distance among ingroup taxa was 0.6% for *rpl32-trnL* sequences, the greatest distance between two ingroup taxa was 2.5% (between *Schefflera*

monophylla 409 and *S. fosbergiana* 3337), while 2,016 pairwise combinations provided distance values of zero.

Sequence lengths for *ndhF-rpl32* ranged from 779 to 1,250 with a total of 1,389 aligned nucleotide characters and 13 coded indels (for a total of 68 parsimony-informative characters). Nucleotide percentages for *ndhF-rpl32* sequences were A = 39.6%, C = 12.6%, G = 12.4%, and T = 35.5%. Average pairwise distance among ingroup taxa was 0.8% for *ndhF-rpl32* sequences, the greatest distance between two ingroup taxa was 2.4% (between *Schefflera staufferana* 7082 and *S. myriantha* 4988), while 639 pairwise combinations provided distance values of zero. Four specimens did not amplify for *ndhF-rpl32* and this missing data was coded as ambiguous (N) (including *Schefflera humblotiana* 3883, *S. stolzii* 3577, *S. barteri* 5208, and *S. barteri* 16998).

Significant incongruence among the three partitions established in this study could not be rejected based on results of the ILD test ($p = 0.01$), suggesting that the partitions may not be combinable. Nonetheless, to explore the effects of combining the datasets, all three partitions were concatenated and analyzed simultaneously. The combined ITS + ETS datasets consisted of 1,149 aligned characters, 198 (≈17.2%) of which were considered parsimony informative.

MP analysis of ETS sequences produced 51,600 total trees, each of 279 steps (CI = 0.675, RI = 0.954, Fig. 1). MP analysis of the ITS dataset produced 96,600 trees, each of 271 steps (CI = 0.708, RI = 0.959, Fig. 2). The combined ITS + ETS dataset produced a total of 100,000 best trees, each of 565 steps in length (CI = 0.665, RI = 0.951, Fig. 3). The greatest resolution among all datasets in this study was provided by this combined ITS + ETS dataset and for this reason the strict consensus tree from this dataset will be used for most of the discussions and for character-state mapping.

Given the low sequence variation of the three plastid markers (all linked on the non-recombining plastid genome), the three plastid spacers were analyzed together. They yielded a total of 3,521 characters, 168 (≈4.7%) of which were parsimony-informative, including 23 indels that were identified as potentially informative and coded as binary characters in the MP analyses (these indels were omitted from model-based analyses). MP analysis of the combined plastid dataset produced 87,700 best trees, each of 633 steps (CI = 0.612 excluding uninformative characters, RI = 0.918, Fig. 4).

The dataset combining all nuclear (ITS + ETS) and plastid sequences yielded a total of 4,693 characters, 366 (≈7.8%) of which were parsimony informative when coded indels were included. MP analysis of the combined nuclear + plastid dataset resulted in 94,200 best trees, each of 1,238 steps in length (CI = 0.608, RI = 0.929, Fig. 5).

Comparison of the 88 models tested in jModeltest resulted in the selection of the TIM + Γ + I model of sequence evolution based on the AIC. Both model-based approaches (ML and BI) produced the same topology with only minor variation in branch support. Likelihood for the ML trees were –2,279.056 for ETS (Fig. 6), –2,612.7117 for ITS (Fig. 7), –5,075.1868 for combined ITS + ETS (Fig. 8), –8,530.1115 for plastid (Fig. 9), and –14,328.1040 for combined nuclear + plastid markers (Fig. 10). No variation among tree topologies was found across the 10 runs conducted using GARLI for any of the datasets. The trees resulting from the Bayesian analyses were highly similar to the topologies from the ML searches (Fig. 11–15).

Morphological Character Mapping: Results of morphological character state mapping are provided in Figures 16–21. Morphological characters were mapped on to the phylogeny

resulting from the ML analysis of the combined nuclear (ITS + ETS) dataset. No clear patterns of morphological evolution emerge as a result of this character mapping.

DIVA: Results from the DIVA analysis favored a two-dispersal scenario, with continental Africa being assigned as ancestral for both subgroups *Neocussonia* and *Astropanax* (Fig. 16).

DISCUSSION

Afro-Malagasy* Schefflera *Clade. This study helps to advance the recommendations set forth by Plunkett et al. (2005), whose findings first illustrated extensive polyphyly in the genus *Schefflera* as currently circumscribed. Due to the size and complexity of *Schefflera*, they suggested that recircumscription of the genus should proceed by testing relationships in each of the five geographically distinct clades individually, beginning with in-depth studies of the smaller clades, including Pacific *Schefflera* (approx. 40-50 spp.), Afro-Malagasy *Schefflera* (approx. 48 spp.), and *Schefflera* § *Schefflera* (8 spp.), and broad surveys of the larger Asian and Neotropical clades (c. 200–400 species each). This paper explores relationships among the African and Malagasy species of *Schefflera* with an enhanced sampling that represents nearly 70% of the species diversity of this clade. This study confirms the monophyly of the species from Africa and Madagascar in a single clade of Afro-Malagasy *Schefflera* and this monophyly is strongly supported (BS = 100%, PP = 1.0). Since the publication of most recent studies of Afro-Malagasy *Schefflera* (Bernardi 1979, Bamps 1974), no fewer than 17 new species have been proposed (Lowry, pers. comm.) and of these, seven new species have been included here. Within the Afro-Malagasy clade, additional interspecific relationships are also evident.

Bernardi's (1969) treatment of three series (*Racemosae*, *Myrianthae*, and *Anticipes*) is not supported, but Frodin's (see Plunkett et al. 2005) suggestion that African and Malagasy *Schefflera* represent two morphologically distinct groups is maintained by the presence of two monophyletic groups corresponding to Frodin's "Sciodaphyllum" and "Meiopanax" (BS = 100%, PP = 1.0). These groups will be referred to as *Astropanax* and *Neocussonia* throughout the rest of this paper. In the discussions below, the infrageneric system developed by Frodin for species belonging to this clade will be used as a point for comparison for results from the current study.

Neocussonia *Clade.* Phylogenetic reconstruction placed 22 of the 48 species of Afro-Malagasy *Schefflera* tested in this study in the *Neocussonia* subclade (Figs. 1–3, 5–8, and 10, BS = 100%, Figs. 11–13 and 15, PP = 1.0). This subclade corresponds closely to the "Meiopanax" group of Baillon (1880) and Frodin (see Plunkett et al. 2005). The generic name *Neocussonia* was first used by Hutchinson (1967) to describe several species of *Schefflera* endemic to Madagascar and this name is applied here because it has taxonomic priority over "Meiopananx" at the generic and subgeneric level. Of the species in *Neocussonia*, only two are African endemics (*S. lukwangulensis* and *S. umbellifera*), while the remaining species are all endemic to Madagascar. Resolution among the species in this clade varies by both markers and, in some cases, by analysis type.

Within *Neocussonia*, two large clades are resolved in most topologies, which are referred to as the "Anticipes" clade and the "Palmate-Vantsilana" clade (Figs. 1–3, 5–8, 10–13, and 15). Both of these clades as well as the remaining, less resolved species in *Neocussonia* are placed in a large polytomy at the base of the clade, sister to *Schefflera umbellifera* (Fig. 3, BS = 66% and

Fig. 15, BS = 0.94). In several cases, different samples of the same species are found in more than one subclade, and this pattern (together with other lines of evidence) suggests the possibility of interspecific hybridization.

Schefflera umbellifera is resolved as sister to the rest of *Neocussonia* in the parsimony trees (Fig. 1, BS = 77%, Fig. 3, BS = 66%, Fig. 6, BS = 62%, Fig. 8, BS = 60%, Fig. 11, PP = 0.69, Fig. 13, BS = 0.81, Fig. 15, BS = 0.94). Morphologically, this species is distinct from others in *Neocussonia* in its leaflet margins, which are serrated margins rather than entire or crennate. Similarities exist between the umbellate inflorescence in *Schefflera umbellifera*, which has long, narrow axes, and the inflorescences of three other species in *Neocussonia* (*S. lukwangulensis*, *S. frodiniana*, and *S. rainaliana*). Geographically, *S. umbellifera* is distinct from other species in *Neocussonia* with the exception of *S. lukwangulensis*, which is also endemic to continental Africa. The two model-based analyses of combined plastid datasets place *S. umbellifera* further within *Neocussonia* but branch support for this placement is low (Fig. 9, BS = 55% & Fig. 14, PP = 0.71).

The "Palmate-Vantsilana" clade is so named because of the shared tendency of palmately compound leaves among its species together with the inclusion of *S. vantsilana*, whose epithet is also the vernacular Malagasy name for most *Schefflera* species. Within this clade, there are several subclades containing more than one species, which is evident in the clades containing specimens identified as *Schefflera longipedicellata* and *S. vantsilana* as well as those combining both *S.* "floretii" and *S.* "vohimenensis". A third species, *S.* "rabenantoandroi", is also found in the "Palmate-Vantsilana" clade with consistently high support (Fig. 3, BS = 86%, Figs. 12, 13, and 15, PP = 1.0). *Schefflera* "rabenantoandroi" has been elevated to the species level by Lowry (pers. comm.) from a variety recognized by Bernardi (*S. vantsilana* var. *litoralis*) due to its

distinctive morphology (e.g., large palmately compound leaflets, long petiole and retuse leaflet apex) and distribution in low-elevation littoral forests (compared to other species of the "Palmate-Vantsilana" clade, which are typically found at altitudes greater than 1,000 m). Bayesian inference places the "Palmate-Vantsilana" clade within a larger monophyletic group (Fig. 15, PP = 0.84) comprising subclades containing *S. macerosa* (PP = 1.0) and other specimens identified as *S. longipedicellata* (PP = 1.0).

In assessing the apparent polyphyly of some of the species of the "Palmate-Vantsilana" clade several factors must be considered, including sympatric distributions and overlapping morphological character states. For example, samples of *S.* "vohimenensis" and *S.* "floretii" appear together in two separate clades with low to moderate branch support (Fig. 3, BS = 62%, Fig. 13, PP = 1.0). These two "hypothesized species"are sympatric but were considered distinct because *Schefflera* "floretii" has palmately compound leaves with 3–5 obdeltoid leaflets, whereas *Schefflera* "vohimenensis" has unifoliate or palmately compound leaves with no more than three leaflets, which are larger, more coriaceous, and have different leaf shapes (obovate) than *S.* "floretii". There are three possible explanations for this polyphyly, (1) hybridization between the two species which may have blurred species distinctions, (2) faulty species circumscriptions that do not correctly capture the full range of character states for each putative species, thus separating them erroneously, or (3) incorrect species identifications for some of the samples included in this study.

Additional polyphyly in the "Palmate-Vantsilana" clade is shown for a subclade including specimens identified as *S. longipedicellata* and *S. vantsilana* (Fig. 3, BS = 68%, Fig. 13, PP = 0.99). All specimens identified as *S. longipedicellata* in this clade come from the same locality, while the specimens identified as *S. vantsilana* cover a much wider geographic range.

Sympatry between the two suggests possible hybridization, or perhaps that the definition for *S. vantsilana* should be expanded to include greater variation in leaflet shape. As currently circumscribed, *S. vantsilana* has larger leaflets than *S. longipedicellata* with a strongly retuse apex and an overall obdeltoid leaf shape.

The other subclade nested within *Neocussonia* is labeled "Anticipes", a reference to the similarities of this clade to the composition of species comprising Bernardi's (1974) series of the same name. This clade is not well resolved in MP analyses but morphological features and BI analyses provide moderate to strong support (Fig. 12, PP = 0.96, and Fig. 15, PP = 0.97). Within "Anticipes", a subclade containing three species, *Schefflera halleana*, *S. favargeri*, and *S.* "*humbertii*", is supported in the majority of topologies, with moderate to strong support (Fig. 3, BS = 60%, Fig. 5, BS = 76%, Fig. 13, PP = 0.96, and Fig. 15, PP = 1.0). Elements of this clade are also supported by plastid data (Figs. 4, 9, and 14). In most trees, the "Anticipes" clade includes six additional species, *Schefflera bojeri*, *S. staufferana*, and *S. antoetrensis* (Figs. 3, 8, 10, 12, 13, 15), as well as *S. capuroniana*, *S. moratii*, and *S. andohahelensis* (Figs. 8, 10, 13, 15). The majority of these species (all but *S. bojeri*) share unifoliolate leaves.

Three proposed new species also fall within the "Anticipes" clade, including *Schefflera* "humbertii", *S.* "andohahelensis", and *S.* "antoetrensis". There does not appear to be sufficient evidence to retain *S.* "humbertii" as separate from *S. favargeri* since these two species are both sympatric and not morphologically distinct, but there is strong support for maintaining the two remaining new species in this clade. *Schefflera* "andohahelensis" is the southernmost species in this clade, with a distribution limited to the Vohimena mountain range near Madagascar's southern coast. This species, along with *S.* "antoetrensis", produces copious amounts of thick, milky latex when cut. *Schefflera* "antoetrensis" is distinct from *S.* "andohahelensis" in its more

limited range to a single locality much further north near Antoetra in central Madagascar. The original collection for this species came from a single specimen collected by Capuron, which was placed in the morphologically similar *S. favargeri*, but Capuron's notation on the specimen regarding the abundance of sap suggested a potential distinction from that species.

The remaining five species in *Neocussonia*, *Schefflera rainaliana*, *S. frodiniana*, *S.* "*purpuristyla*", *S. bracteolifera*, and *S. lukwangulensis*, are not well resolved in either the "Anticipes" or "Palmate-Vantsilana" clades. *Schefflera rainaliana* and *S. frodiniana* are resolved together in most analyses (Fig. 3, BS = 70%, Fig. 8, BS = 84%, Fig. 13, PP = 1.0). Both *S. rainaliana* and *S. frodiniana* have an umbellate inflorescence with long, narrow axes, although *S. rainaliana* is unifoliolate and *S. frodiniana* has palmately compound with 3–5 leaflets. Similarly, *S. bracteolifera* and *S.* "purpuristyla" are resolved together in many topologies (Fig. 3, BS = 78%, Fig. 8, BS = 87%, Fig 13, PP = 1.0). The formation of a clade between *S. bracteolifera* and *S.* "purpuristyla" is surprising as the two species differ considerably in their morphologies. *S. bracteolifera* is unifoliolate, while *S.* "purpuristyla" is palmately compound with 5–7 leaflets.

Astropanax *clade.* Ten species were placed in the *Astropanax* clade (Figs. 1–3 and 5–10, BS = 100%, Figs. 11–13 and 15, PP = 1.0) and of these, all but three are endemic to continental Africa. This clade corresponds closely to the African elements of Frodin's "Sciodaphyllum", but Plunkett et al. (2005) demonstrated that "Sciodaphyllum" was polyphyletic. Frodin (see Plunkett et al. 2005) recognized the group on the basis of a "generalized" morphology that includes terminal paniculate inflorescences, leaves crowded at the end of stems, limited branching, and non-ruminate endosperm, features shared among many geographically diverse species of *Schefflera* in Africa, Asia, and the Neotropics and also across many other Araliaceae. Before

being transferred to *Schefflera*, several of these species were placed in the genus *Astropanax* by Seemann (1865). Because the type species of "Sciodaphyllum" is placed among species in the Neotropical *Schefflera*, Afro-Malagasy species of this group are referred to using Seemann's original *Astropanax*. Of the Afro-Malagasy species that Frodin placed in "Sciodaphyllum", only *S. moratii* does not fall in the *Astropanax* clade, but is placed instead among the species of the *Neocussonia* clade. This finding is consistent with the morphological characteristics of *S. moratii*, which has a racemose inflorescence arrangement and strictly unifoliolate leaves.

Perhaps most surprising in this clade is the paraphyly of *Schefflera myriantha* with respect to two Malagasy species, *S. monophylla* and *S. humblotiana*. Of these, *S. monophylla* represents the most morphologically diverse species in this clade, but is geographically restricted to Madagascar. Conversely, *S. myriantha* has the broadest geographic range (continental Africa, Comoro islands, and Madagascar) among species of Afro-Malagasy *Schefflera*, but morphologically, the specimens from Africa are nearly indistinguishable from those distributed throughout the Comoros and Madagascar. Both the separate and combined analyses of the nuclear and plastid datasets indicate paraphyly in *S. myriantha* with strong branch support (Fig. 3, BS = 96%, Fig. 8, BS = 95%, Figs. 12–15, PP = 1.0). Lower branch support in plastid and combined nuclear + plastid topologies appears to result from inconsistent placement of a single specimen of *S. monophylla* (Fig. 5, BS = 58%, Fig. 10, BS = 65%). The lack of morphological differences in *S. myriantha* from Africa *vs.* Madagascar suggests the need for a more comprehensive study of specimens in light of the molecular divergence demonstrated by this study. The morphological distinction between *S. monophylla* and *S. humblotiana* is clear. *Schefflera monophylla*, despite its epithet, is typically not truly unifoliolate, but instead has a large central leaflet with two much smaller lateral leaflets that are sometimes scarcely evident

but only rarely absent. Morphologically, *S. humblotiana* is the most distinctive of these three species, possessing extremely long, narrow leaflets. Due to the molecular divergence among species belonging to what are labeled the "*myriantha-monophylla*" and "African *myriantha*" clades, African *S. myriantha* may warrant recognition as a new species, but this decision must be delayed until specimens from the Comoros (currently lacking) can be included in a formal study.

There is strong support for another clade in *Astropanax* referred to as the "Goetzenii" clade, which includes three species, *Schefflera goetzenii*, *S. barteri*, and *S. tessmannii* (Fig. 5, BS = 96%, Figs. 13 and 15, PP = 1.0). Resolution is poor within the clade, and while there are subclades, they vary in topology across datasets. In some cases, *S. goetzenii* is sister to *S. barteri* and *S. tessmannii* (Fig. 5, BS = 80%), while other trees place specimens of each species in a polytomy (Fig. 3, BS = 90%, Figs. 13 and 15, PP = 1.0). Each of the three species in the "Goetzenii" clade is highly distinct morphologically. *Schefflera goetzenii* has caducous bracts and flowers with six-carpellate ovaries, palmately compound leaves with 6–7 narrowly obovate leaflets and a racemose inflorescence arrangement. Both *S. barteri* and *S. tessmannii* have long, persistent bracts but differ in carpel number (*S. barteri* is 7–9 carpellate, *S. tessmannii* is 5–6 carpellate) and number of leaflets (*S. barteri* has 5–8 leaflets, *S. tessmannii* has 6-8 leaflets), however, *S. barteri* and *S. tessmannii* both share inflorescence arrangement features with *S. goetzenii* with a paniculate-racemenose inflorescence. Geographically these species represent a large portion of tropical Africa from western sub-Saharan Africa and São Tomé to east-central sub-Saharan Africa. Poor sampling in this clade is likely attributable to the lack of resolution.

The remaining four species in the *Astropanax* clade form a basal polytomy with the *Schefflera myriantha-S. monophylla* complex and the "Goetzenii" clade, and include *S. volkensii*, *S. mannii*, *S. kivuensis* and *S. stolzii*. Differences in resolution among species belonging to

Astropanax may be due to missing data from *ndhF-rpl32* for *Schefflera humblotiana*, *S. stolzii*, and two samples of *S. barteri*. On the basis of morphology (and to some degree geography) several additional species may also belong to *Astropanax*, but these species (*Schefflera abyssinica*, *S. evrardii*, *S. hierniana*, *S. procumbens*, *S. stuhlmannii*, and *S. urostachya*) were not available for sampling. Despite this limitation, sampling of *Astropanax* relative to Frodin's Afro-Malagasy "Sciodaphyllum" resulted in ≈62% species coverage and differed only in the placement of *Schefflera* "*moratii*".

Geography. Results of the DIVA study suggests an African origin for the entire Afro-Malagasy *Schefflera* clade and an African origin for both the *Neocussonia* and *Astropanax* clades (Fig. 16). In *Neocussonia*, the African species, *Schefflera umbellifera*, is sister to the remaining members of the clade. A second *Neocussonia* species, *S. lukwangulensis*, is also endemic to continental Africa, but its placement in a polytomy renders biogeographic inference equivocal. Two scenarios are possible – either a single divergence from continental Africa to Madagascar or a divergence to Madagascar followed by a secondary dispersal back to Africa. In *Astropanax*, the ancestral area is continental Africa. A single divergence event in *Astropanax* appears to have led to the presence of *S. humblotiana*, *S. monophylla* and Malagasy representatives of *Schefflera myriantha*. Conclusions regarding biogeographic hypotheses for the Comoro Islands were not possible due to the unavailability of material representing specimens of *S. myriantha* from the Comoros.

Morphological patterns. All species in the *Neocussonia* clade share either 2–3 or 4–5-carpellate ovaries, whereas species in the *Astropanax* clade share 4–5 or 6 or more carpels (Fig.

17). A trend towards increases in carpel number is evident in each clade. In *Astropanax* there is a single increase from the ancestral state of 4–5 carpels to 6 or more carpels, but this is restricted to the "Goetzenii" clade. In *Neocussonia*, there are multiple independent increases in carpel number from 2–3 (the ancestral state) to 4–5 carpels in *S. staufferana,* in *S. frodiniana,* and again in *S. moratii*. Palmately compound leaves are ancestral in both *Neocussonia* and *Astropanax*, with reduction in leaflet number arising independently in each clade. There is a single reduction (*S. monophylla*) in *Astropanax* (Fig. 18), but several reductions of leaflet number in the *Neocussonia* clade. There are nine instances of leaflet reduction from plurifoliolate to unifoliolate leaves, and in at least one case what appears to be reduction to simple leaves due to loss of the leaflet articulation between the petiole and the petiolule (*S. antoetrensis*). Inflorenscence structure is highly labile in both *Neocussonia* and *Astropanax* (Fig. 19). Umbellate inflorence arrangement appears ancestral in *Neocussonia*, with racemose inflorenscences common among several species. Species in *Astropanax* have a primarily racemose inflorescence arrangement with the large exception found in the "African *myriantha*" and "*myriantha-monophylla*" clades. Three species in *Astropanax* (*Schefflera stolzii*, *S. volkensii*, and *S. mannii*) possess spikes, having completely lost the pedicels in their flowers, and this trait is shared with outgroup members in the genus *Cussonia*. Other species in *Astropanax* have longer pedicels, primarily among species of *S. myriantha* and *S. monophylla* (Fig. 20). Species in *Neocussonia* appear to have undergone several reductions in pedicel length, although no species in this clade have completely lost this character. The persistence or loss of bracts in mature inflorescences is also highly labile in both *Neocussonia* and *Astropanax* (Fig. 21).

Conclusions. All phylogenic trees produced as a result of this study confirm previous findings of monophyly among species of Afro-Malagasy *Schefflera* (Plunkett et al. 2005). The study of this clade represents part of a larger effort to recircumscribe all five clades that comprise the polyphyletic genus *Schefflera*, and a parallel effort to recircumscribe *Polyscias*, the second largest genus in Araliaceae. However, unlike *Schefflera*, which is polyphyletic, *Polyscias* is paraphyletic with respect to six other genera, and thus it has been expanded to include all the species from *Arthrophyllum*, *Cuphocarpus*, *Gastonia*, *Munroidendron*, *Reynoldsia*, and *Tetraplasandra* (Plunkett & Lowry 2010, Lowry & Plunkett 2010). By contrast, the polyphyly of *Schefflera* will ultimately require the recognition of several separate genera. Only the species belonging to the small *Schefflera* § *Schefflera* clade (8 spp.) will retain that generic name, while the species belonging to the remaining four clades in *Schefflera* will require taxonomic transfers to several new or reinstated genera. The task of recircumscribing species belonging to Afro-Malagasy *Schefflera* could proceed in one of two ways, either recognition of a single genus comprising all species in the clade, or two separate genera comprising species assigned to either *Neocussonia* or *Astropanax*.

Future studies should focus on sampling from the 16 species left unsampled here and perhaps a morphometric approach to delimiting species in the Afro-Malagasy *Schefflera* clade, particularly for difficult species-complexes, including several in the *Astropanax* clade (e.g., *Schefflera humblotiana*, *S. monophylla*, and *S. myriantha*) and species belonging to the "Palmate-Vantsilana" clade in *Neocussonia*. The present study provides the foundation for such future work, and makes a considerable contribution to the realignments necessary in both *Schefflera* sensu lato and Araliaceae.

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TABLE 1: Species used for DNA samples used in this study, including geographic range and voucher information.

Taxon	Geographic locality	Source and accession no.
Outgroups		
Astrotricha pterocarpa Benth.	Australia	Queensland, Australia Plunkett 1527 (NY)
Cussonia holstii Harms ex. Engl.	Southern Africa	Tanzania, Lowry 4986 (MO)
Cussonia paniculata Eckl. & Zeyh.	Southern Africa	South Africa, Phillipson 5263 (MO)
Cussonia thyrsiflora Thunb.	Southern Africa	South Africa, Phillipson 5110 (MO)
Osmoxylon pectinatum (Merr.) Phillipson	Taiwan	Green Is., Taiwan Huang 756 (HAST)
Schefflera digitata J.R. Forst. & G. Forst.	New Zealand	North Island, New Zealand Plunkett 2190 (NY)
Seemannaralia gerrardii (Seem.) R. Vig.	South Africa	South Africa, Phillipson 5471 (MO)
Ingroup, *Neocussonia* subclade		
Schefflera "andohahelensis" Lowry ined.	Madagascar	Andohahela, Madagascar RBE 1505 (MO) Ivorona, Madagascar Lowry 7164 (MO) Ivorona, Madagascar Lowry 7169 (MO) Ivorona, Madagascar Lowry 7171 (MO)
Schefflera "antoetrensis" Lowry ined.	Madagascar	Antoetra, Madagascar Lowry 7088 (MO) Antoetra, Madagascar Lowry 7091 (MO)
Schefflera bojeri (Seem.) R. Vig.	Madagascar	Fianarantsoa, Madagascar Lowry 5818 (MO) Antsirabe, Madagascar Lowry 7071 (MO) Antsirabe, Madagascar Lowry 7109 (MO)
Schefflera bracteolifera Frodin	Madagascar	Mahajanga, Madagascar Lowry 5327 (MO)
Schefflera capuroniana (Bernardi) Bernardi	Madagascar	Antananarivo, Madagascar Bernardi 11118 (P) Ankazobe, Madagascar Plunkett 2328 (MO) Ambatovy, Madagascar Gostel 33 (MO)
Schefflera favargeri Bernardi	Madagascar	Mahajanga, Madagascar Callmander 384 (MO) Mahajanga, Madagascar Callmander 444 (MO) Antsiranana, Madagascar Ratovoson 476 (MO) Mahajanga, Madagascar Lowry 5344A (MO)
Schefflera "floretii" Lowry ined.	Madagascar	Ivorona, Madagascar Lowry 7160 (MO) Ivorona, Madagascar Lowry 7162 (MO)

		Ivorona, Madagascar Lowry 7172 (MO)
		Andohahela, Madagascar RBE 1485 (MO)
		Andohahela, Madagascar RBE 1487 (MO)
		Ivorona, Madagascar RZK 4558 (MO)
		Ivorona, Madagascar RZK 4941 (MO)
Schefflera fosbergiana (Bernardi) Bernardi	Madagascar	Antsiranana, Madagascar Schmidt 4322 (MO)
		Marojejy, Madagascar RD 3350 (MO)
		Makirovana, Madagascar RD 3337 (MO)
Schefflera frodiniana Bernardi	Madagascar	Andohahela, Madagascar RBE 1486 (MO)
Schefflera halleana Bernardi	Madagascar	Antsiranana, Madagascar Schmidt 4261 (MO)
		Marojejy, Madagascar RD 3339 (MO)
		Marojejy, Madagascar RD 3340 (MO)
		Marojejy, Madagascar RD 3343 (MO)
Schefflera "humbertii" Lowry ined.	Madagascar	Antsiranana, Madagascar Lowry 5386 (MO)
Schefflera longipedicellata (Lecomte) Bernardi	Madagascar	Toamasina, Madagascar Lowry 6153 (MO)
		Toamasina, Madagascar Lowry 6220 (MO)
		Antananarivo, Madagascar Lowry 6264 (MO)
		Antoetra, Madagascar Lowry 7098 (MO)
		Antoetra, Madagascar Lowry 7104 (MO)
		Ambatovy, Madagascar Gostel 21 (MO)
		Ambatovy, Madagascar Gostel 23 (MO)
		Ambatovy, Madagascar Gostel 33 (MO)
Schefflera lukwangulensis (Tennant) Bernardi	Tanzania	Mabberly 1197 (MO)
Schefflera macerosa Bernardi	Madagascar	Andohahela, Madagascar RBE 1499 (MO)
		Andohahela, Madagascar RBE 1509 (MO)
Schefflera moratii Bernardi	Madagascar	Antsevabe, Madagascar Gostel 31 (MO)
		Antsevabe, Madagascar Gostel 34 (MO)
Schefflera "purpuristyla" Lowry ined.	Madagascar	Mahajanga, Madagascar Callmander 432 (MO)
Schefflera "rabenantoandroi" Lowry ined.	Madagascar	Tolagnaro, Madagascar Lowry 7148 (MO)
		Tolagnaro, Madagascar Lowry 7149
Schefflera rainaliana Bernardi	Madagascar	Tolagnaro, Madagascar McPherson 14791 (MO)
		Tolagnaro, Madagascar Lowry 4444 (MO)

		Tolagnaro, Madagascar Lowry 7151 (MO)
Schefflera staufferana Bernardi	Madagascar	Antsiranana, Madagascar McPherson 17219 (MO)
		Toamasina, Madagascar Ratovoson 170 (MO)
		Ambatovy, Madagascar Gostel 24 (MO)
		Ambatovy, Madagascar Gostel 25 (MO)
		Ambatovy, Madagascar Gostel 26 (MO)
		Antoetra, Madagascar Lowry 7082 (MO)
Schefflera umbellifera (Sond.) Baillon	Southern Africa	S. Africa, Goldblatt 11950 (MO)
		Phillipson 5494 (MO)
Schefflera vantsilana (Bak.) Bernardi	Madagascar	Toamasina, Madagascar Lowry 6225 (MO)
		Toamasina, Madagascar Randrianasolo 152 (MO)
		Ambatovy, Madagascar Gostel 32 (MO)
		Antoetra, Madagascar Lowry 7103 (MO)
Schefflera "vohimenensis" Lowry ined.	Madagascar	Ivorona, Madagascar Plunkett 2378 (MO)
		Bemangedy, Madagascar Lowry 7156 (MO)
		Ivorona, Madagascar CR 4710 (MO)
Ingroup, *Astropanax* subclade		
Schefflera barteri (Seem.) Harms	Western sub-Saharan Africa and São Tomé	Cameroon, Etuge 5208 (P)
		Gabon, McPherson 16998 (P)
		Cameroon, Thomas 6782 (NY)
Schefflera goetzenii Harms	Central and Eastern sub-Saharan Africa	Zimbabwe, Lowry 4807 (MO)
		Rwanda, Auquier 3503 (BR)
		D.R. Congo, Cephas 358 (BR)
Schefflera humblotiana Drake	Madagascar	Toamasina, Madagascar Schatz 3883 (P)
Schefflera kivuensis P. Bamps	Central sub-Saharan Africa	Belgian Congo, Gutzwiller 2145 (BR)
Schefflera mannii (Hook. f.) Harms	Western sub-Saharan Africa	Equitorial Guinea, Cavalho 4406 (BR)
		?, s.n. ?
Schefflera monophylla (Baker) Bernardi	Madagascar	Antsiranana, Madagascar Randrianasolo 409 (MO)
		Toamasina, Madagascar Randrianasolo 131 (MO)

		Antoetra, Madagascar Lowry 7087 (MO) Antoetra, Madagascar Lowry 7097 (MO) Ivorona, Madagascar Lowry 7173 (MO) Andohahela, Madagascar RBE 1492 (MO)
Schefflera myriantha (Baker) Drake	Eastern sub-Saharan Africa, Comoro Islands, & Madagascar	Tanzania, Lowry 4988 (MO) Antananarivo, Madagascar Lowry 5808 (MO) Antananarivo, Madagascar Lowry 5812 (MO) Antananarivo, Madagascar Lowry 5816 (MO) Mahajanga, Madagascar Lowry 5347 (MO) Mahajanga, Madagascar Lowry 5445 (MO) Anjavokely, Madagascar Lowry 5796 (P) Anjavokely, Madagascar Lowry 5798 (MO) Toamasina, Madagascar Lowry 6117 (MO) Tanzania, Mwangulango 501 (MO) Rwanda, P. Bamps 3050 (BR) Ethiopia, Danish-Ethiopian Exp. 1885 (BR) Malawi, Van der Linden L365 (BR) Antsiranana, Madagascar Ratovoson 470 (MO) Andohahela, Madagascar RBE 1498 (MO)
Schefflera stolzii Harms	Central-Eastern sub-Saharan Africa	Gereau 3577 (P)
Schefflera tessmannii Harms	Western sub-Saharan Africa	Gabon, Reitsma 2107 (P)
Schefflera volkensii (Engl.) Harms	Eastern sub-Saharan Africa	Tanzania, Lowry 4987 (MO) Tanzania, Phillipson 5129 (P)
Schefflera sp.	Madagascar	Makirovana, Madagascar RD 3332 (MO) Makirovana, Madagascar RD 3333 (MO) Makirovana, Madagascar RD 3334 (MO) Makirovana, Madagascar RD 3335 (MO) Makirovana, Madagascar RD 3336 (MO) Marojejy, Madagascar RD 3342 (MO) Marojejy, Madagascar RD 3344 (MO) Marojejy, Madagascar RD 3345 (MO) Marojejy, Madagascar RD 3347 (MO) Marojejy, RD 3348 (MO)

	Marojejy, RD 3349 (MO) Marojejy, RD 3351 (MO) Marojejy, RD 3352 (MO)

TABLE 2: Oligonucleotide primers used for PCR amplification and DNA sequencing.

Primer Name	Primer Region	Primer Sequence (5' – 3')	Primer Length	Citation:
Nuclear:				
ITS5-F	ITS	GGA AGT AAA AGT CGT AAC AAG G	22 bp	White et al., 1990
C26A-R	ITS	TTT CTT TTC CTC CGC T	16 bp	Wen and Zimmer, 1996
ETS-400F	ETS	GTT GGT CGG ATC CCT GCT TGT	21 bp	Fiaschi & Plunkett (in pres
18S-2L (-r)	ETS	TGA CTA CTG GCA GGA TCA ACC AG	23 bp	Linder et al., 2000
Plastid:				
ndhF-rpl32-F	*ndhF-rpl32* IGS	GCA TAT TGA TAT GTC TGT TCC AT	23 bp	Plunkett (unpubl.)
ndhF-rpl32-R	*ndhF-rpl32* IGS	AAG AGA TTT CCC TAA TGA CAA CGC	24 bp	Plunkett (unpubl.)
ndhF-rpl32-MF	*ndhF-rpl32* IGS	GAG TAC TTG ATT CTG ATA TGA ATC	24 bp	Gostel (unpubl.)
ndhF-rpl32-MR	*ndhF-rpl32* IGS	AGA ATC CGC CGT TAT GCC ATA G	22 bp	Gostel (unpubl.)
trnK-(rps16)_NF	*trnK-rps16* IGS	AGC CGA GTA CTC TAC CGT TGA	21 bp	Nicolas (unpubl.)
(trnK)-rps16_NR	*trnK-rps16* IGS	GAG CCG TCT ATC GAA TCG TTG CAA	24 bp	Nicolas (unpubl.)
trnK-rps16-MF	*trnK-rps16* IGS	GCA CGG AAA TAA ATC GAT CCG C	22 bp	Plunkett (unpubl.)
trnK-rps16-MR	*trnK-rps16* IGS	CGT TGG AAC TTT ACT AAC ACG	21 bp	Plunkett (unpubl.)
rpl32/trnL_F	*rpl32-trnL* IGS	GCG TTG TCA TTA GGG AAA TCT CTT	24 bp	??
rpl32/trnL_R	*rpl32-trnL* IGS	GCT TCC TAA GAG CAG CGT GTC T	22 bp	??
rpl32-trnL MF	*rpl32-trnL* IGS	CTA TCT CTA TAT CTA TAG AAG GGC AAA	27 bp	Gostel (unpubl.)
rpl32-trnL MR	*rpl32-trnL* IGS	TGA TTG GGT TGA AAG CAG GAG ATA TAT GGG AGA	38 bp	Gostel (unpubl.)

FIGURE LEGENDS

Fig. 1. The strict consensus of 51,600 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the nuclear ETS rDNA spacer. Tree length = 279 steps, CI = 0.675, RI = 0.954. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 2. The strict consensus of 96,600 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the nuclear ITS rDNA spacer. Tree length = 271 steps, CI = 0.708, RI = 0.959. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 3. The strict consensus of 100,000 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Tree length = 565 steps, CI = 0.665, and RI = 0.951. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 4. The strict consensus of 87,700 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Tree length = 655 steps, CI = 0.612, RI = 0.918. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are

provided above the branches; bootstrap values less than 50% are recorded as "<". Gray arrows indicate specimens whose placement has moved considerably from other analyses.

Fig. 5. The strict consensus of 94,200 trees resulting from the maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Tree length = 1,238 steps, CI = 0.608, RI = 0.929. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. Parsimony bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 6. The best tree (log likelihood = –2,279.056) based on maximum likelihood (ML) analysis of 123 sequences from the nuclear ETS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 7. The best tree (log likelihood = –2,612.7117) based on maximum likelihood (ML) analysis of 123 sequences from the nuclear ITS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 8. The best tree (log likelihood = –5,075.1868) based on maximum likelihood (ML) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap

percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 9. The best tree (log likelihood = –8,530.1115) based on maximum likelihood (ML) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<". Gray arrows indicate specimens whose placement has moved considerably from other analyses.

Fig. 10. The best tree (log likelihood = –14,328.1040) based on maximum likelihood (ML) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. ML bootstrap percentages are provided above the branches; bootstrap values less than 50% are recorded as "<".

Fig. 11. The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the nuclear ETS rDNA spacer. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches.

Fig. 12. The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the nuclear ITS rDNA spacer. Brackets to the right of taxon labels correspond to

informal clade names discussed in the text. BI posterior probabilities are provided above the branches.

Fig. 13. The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches.

Fig. 14. The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches. Gray arrows indicate specimens whose placement has moved considerably from other analyses.

Fig. 15. The majority-rule tree based on the Bayesian-inference (BI) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers and combined plastid markers, *trnK-rps16*, *rpl32-trnL*, and *ndhF-rpl32*. Brackets to the right of taxon labels correspond to informal clade names discussed in the text. BI posterior probabilities are provided above the branches.

Fig. 16. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with geographic locality mapped onto branches using MacClade (version 4.08). DIVA (version 1.1a) output is provided

above branches to indicate ancestry for divergence nodes. “A” corresponds to continental Africa and “B” corresponds to Madagascar.

Fig. 17. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with carpel number states mapped onto branches.

Fig. 18. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with leaf composition character states mapped onto branches.

Fig. 19. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with inflorescence arrangement states mapped onto branches.

Fig. 20. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with pedicel lengths mapped onto branches.

Fig. 21. The strict consensus tree resulting from maximum parsimony (MP) analysis of 123 sequences from the combined nuclear ITS + ETS rDNA spacers with bract persistence mapped onto branches.

Figure 1: MP, ETS

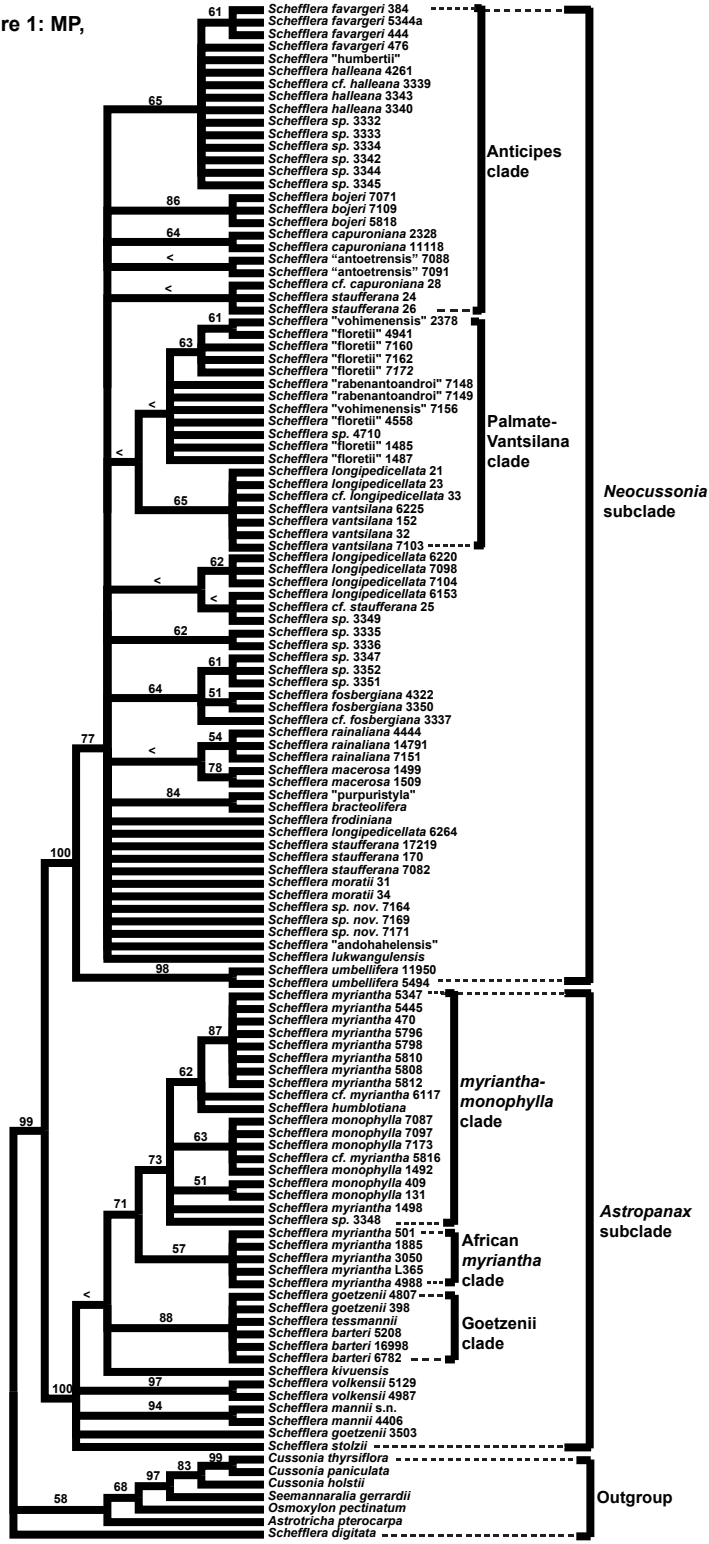


Figure 2: MP, ITS

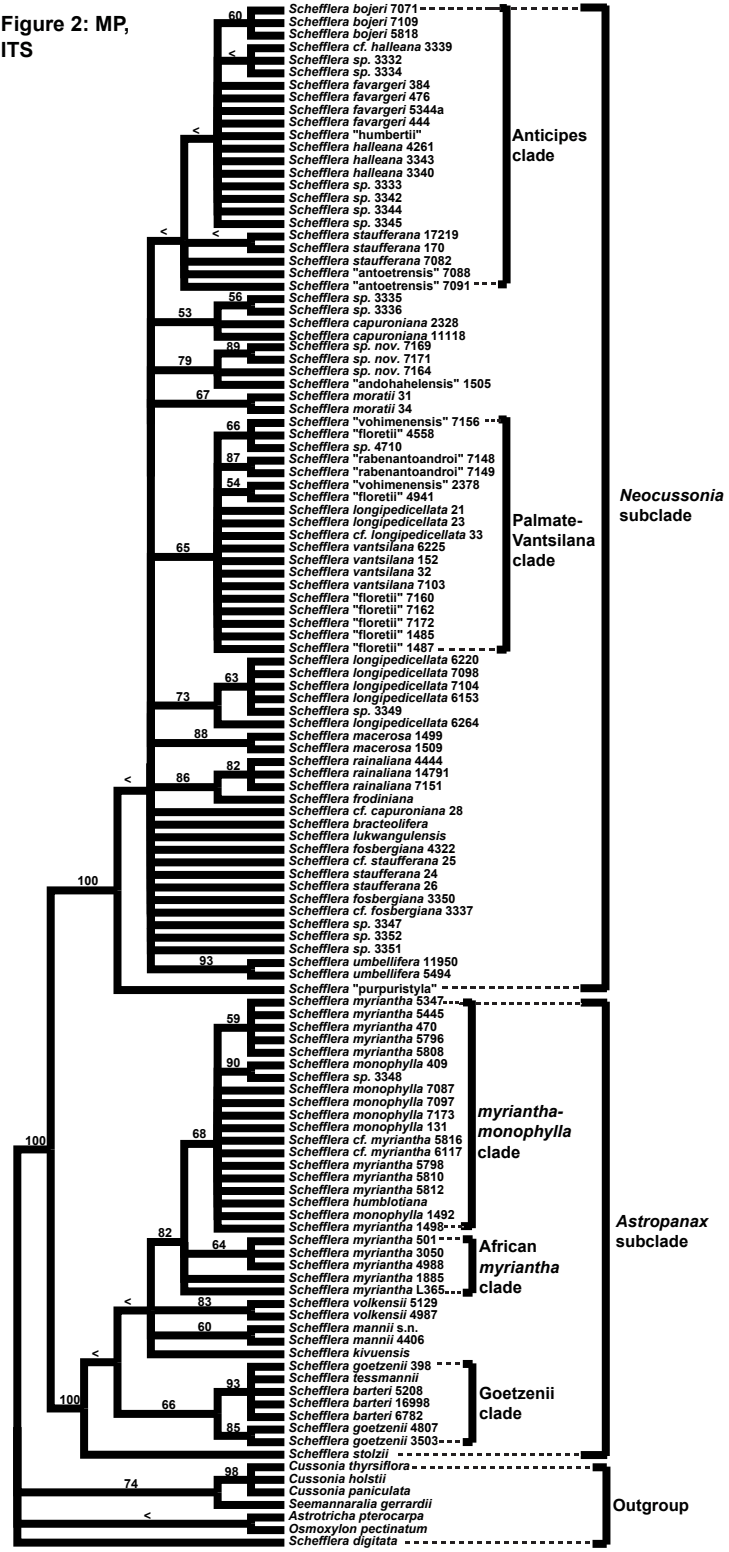


Figure 3: MP, Nuclear, combined

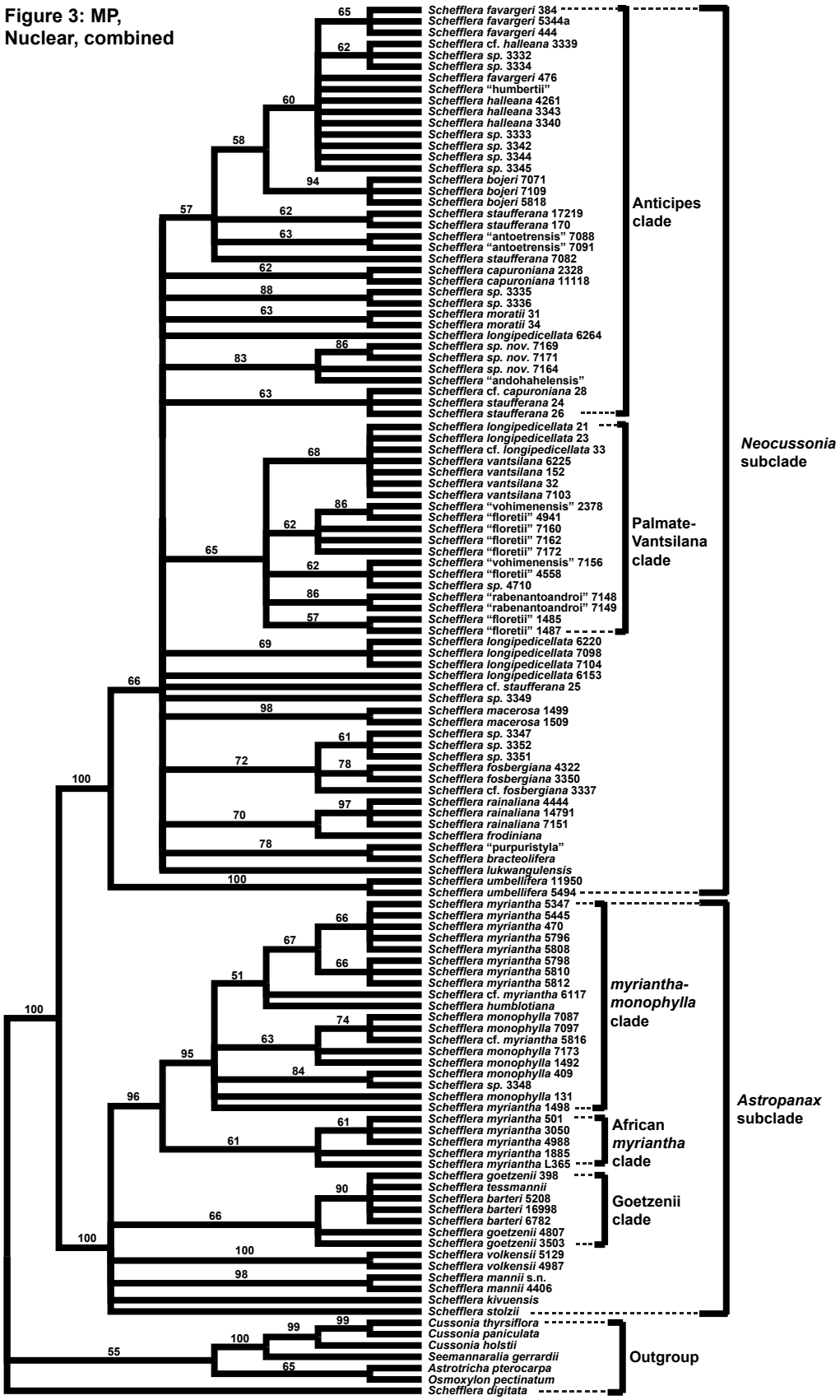


Figure 4: MP, Plastid, combined

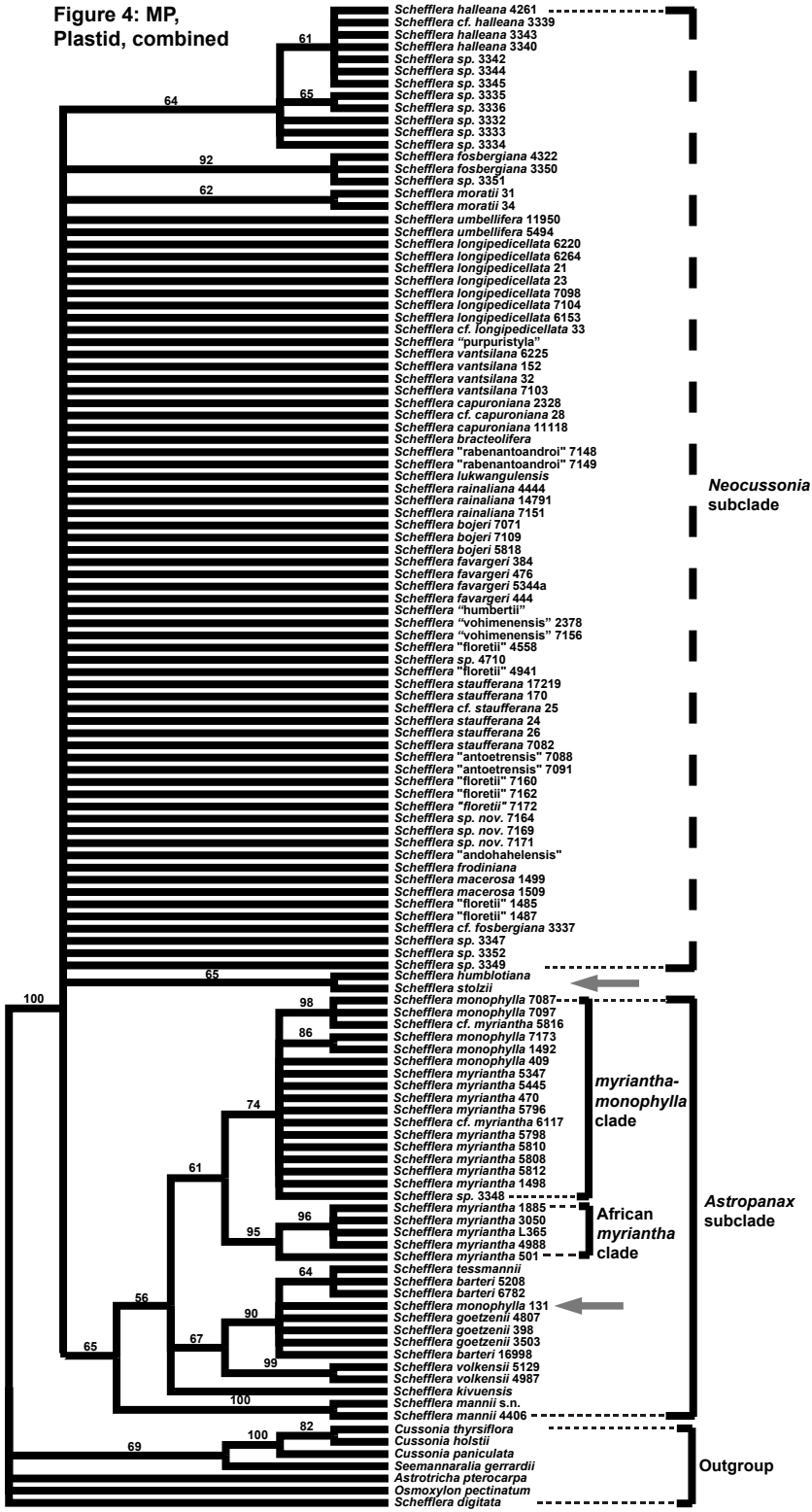


Figure 5: MP, Nuclear + Plastid, combined

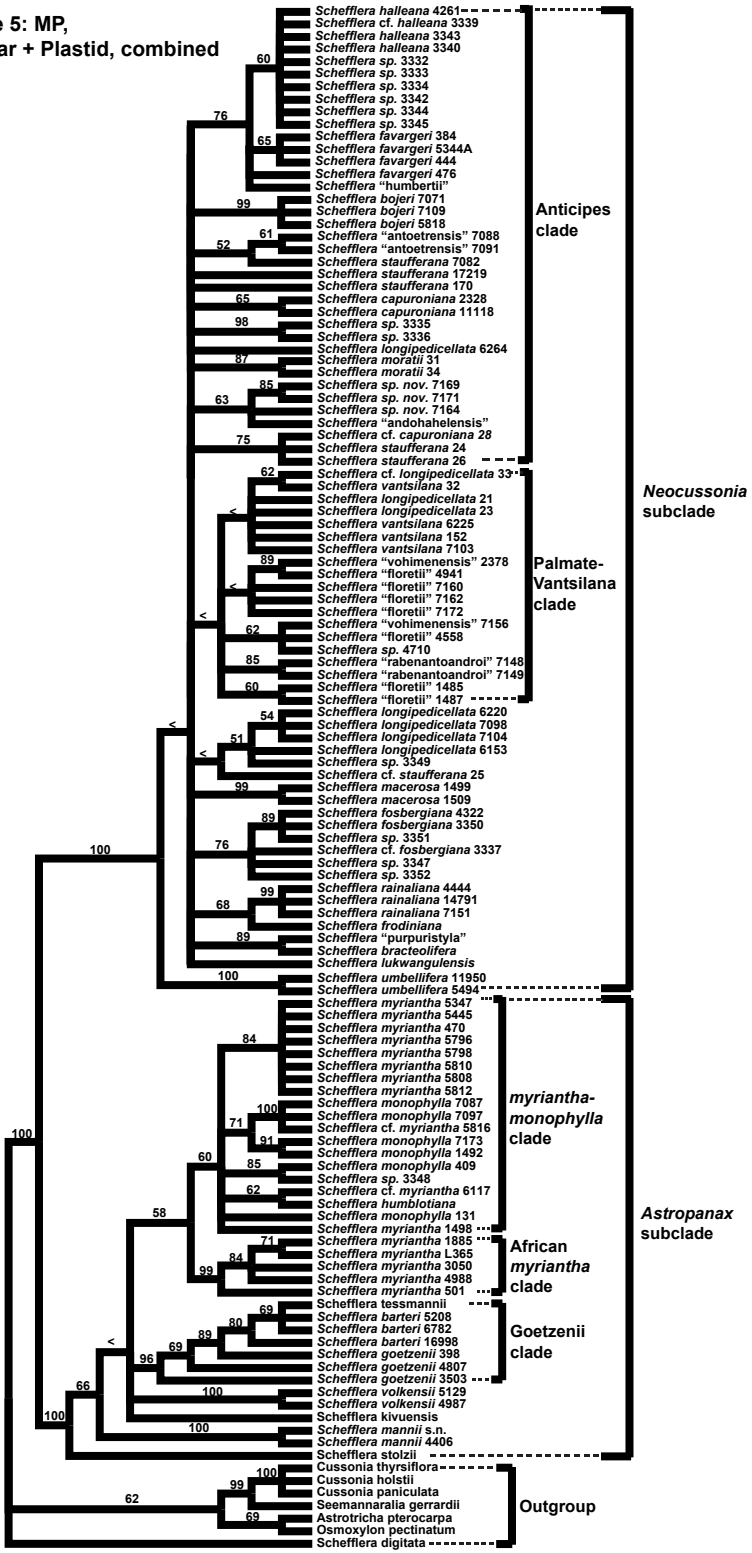


Figure 6: ML, ETS

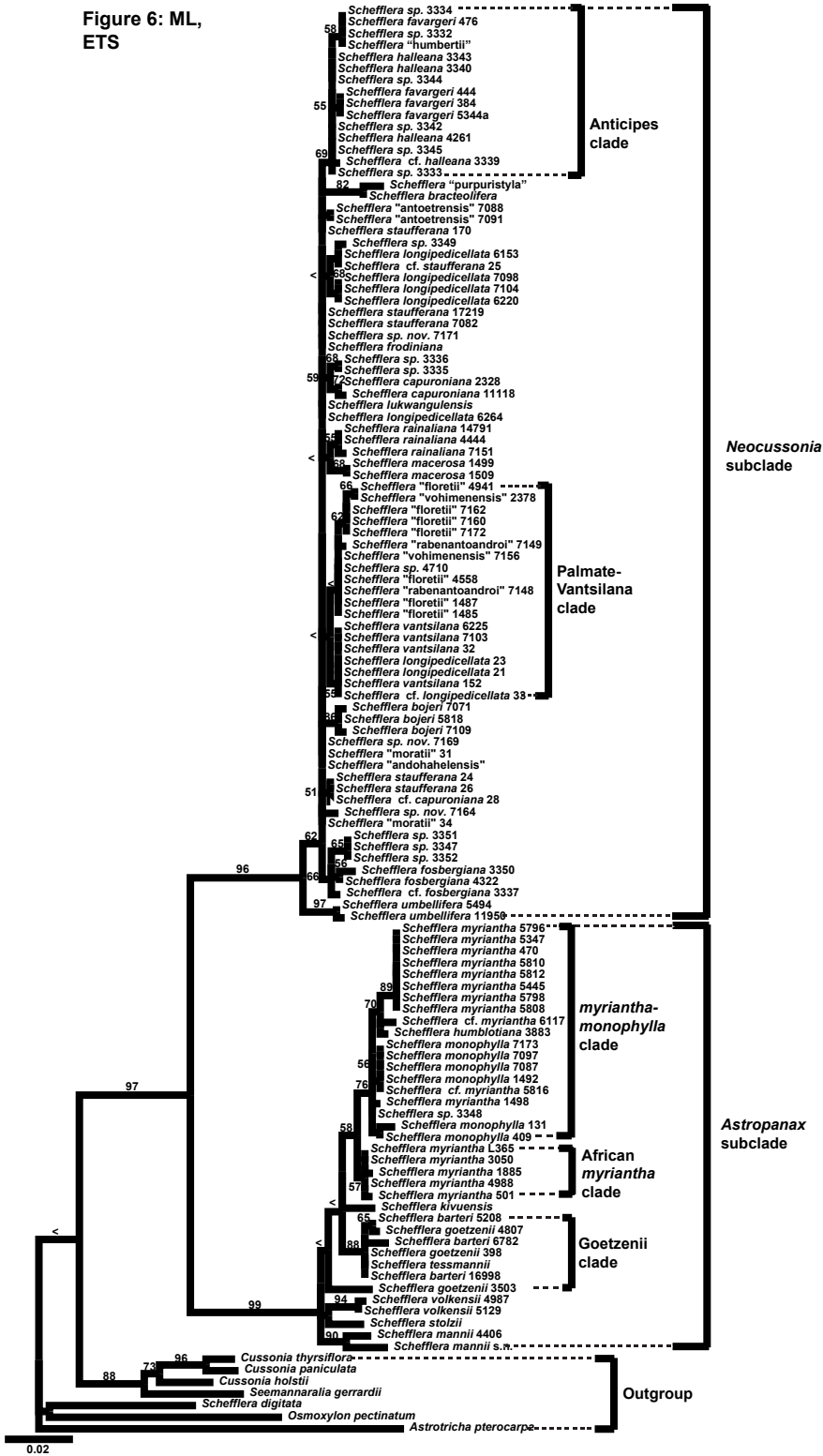


Figure 7: ML, ITS

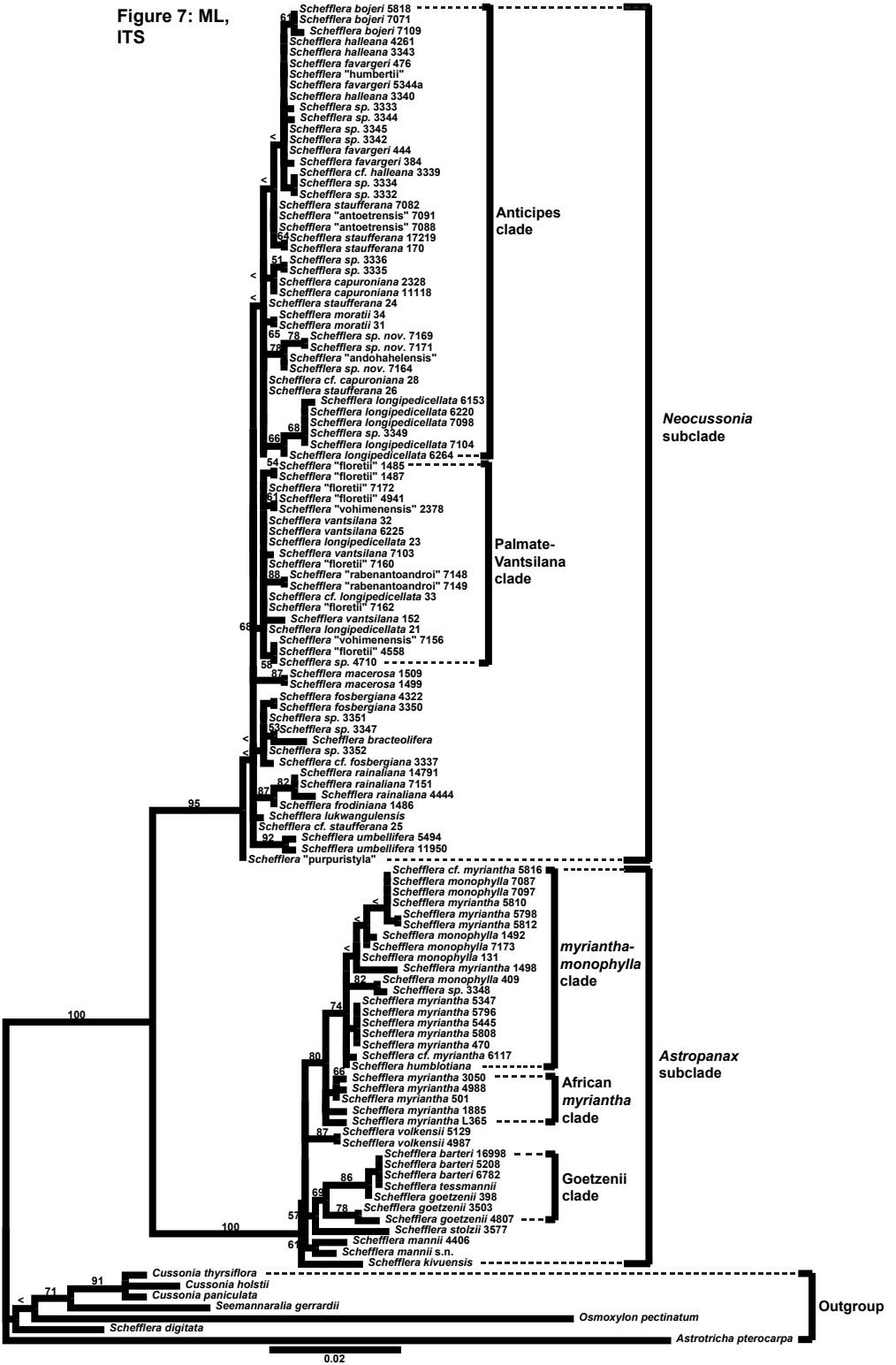


Figure 8: ML, Nuclear, combined

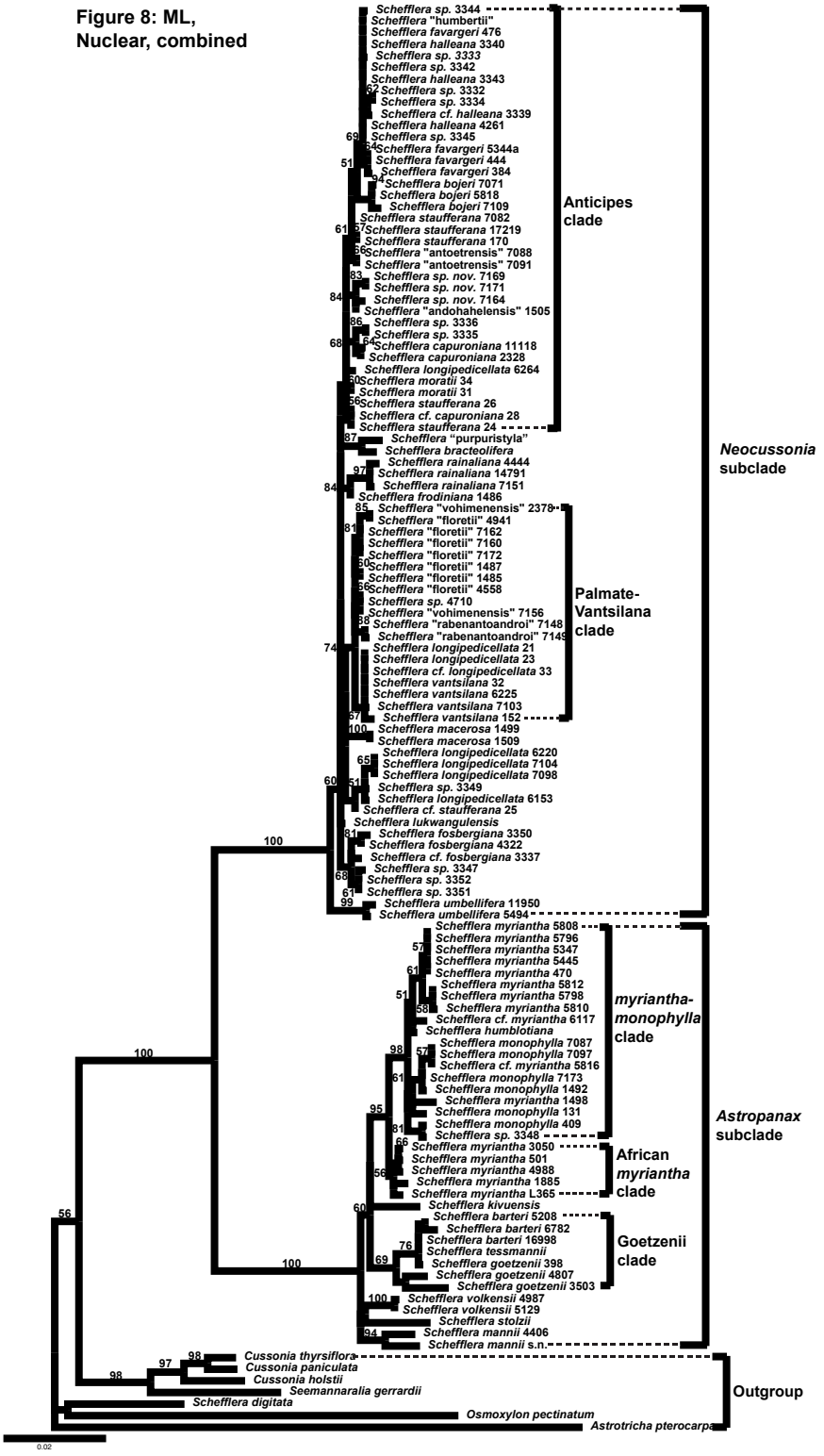


Figure 9: ML, Plastid, combined

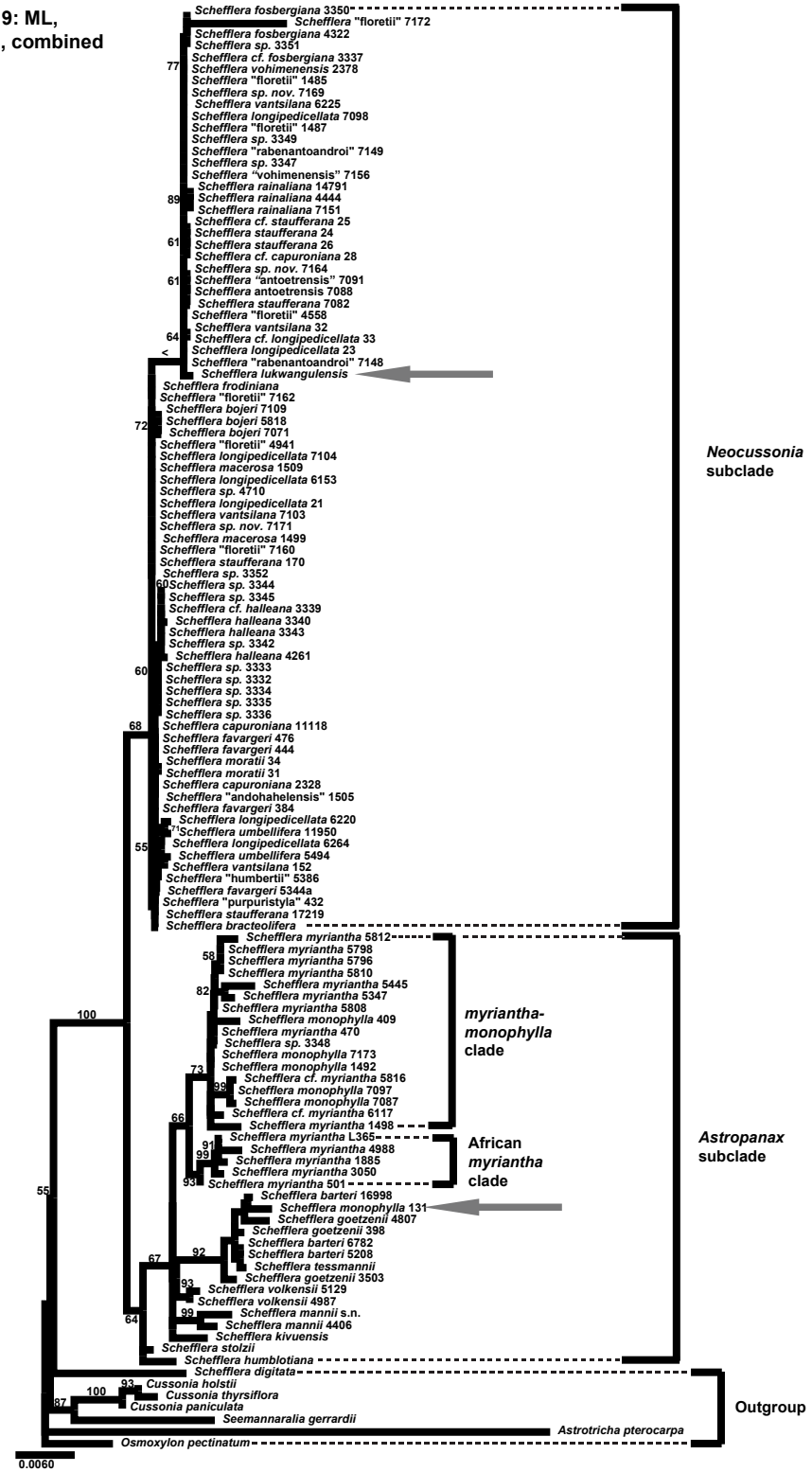


Figure 10: ML, Nuclear + PLastid, combined

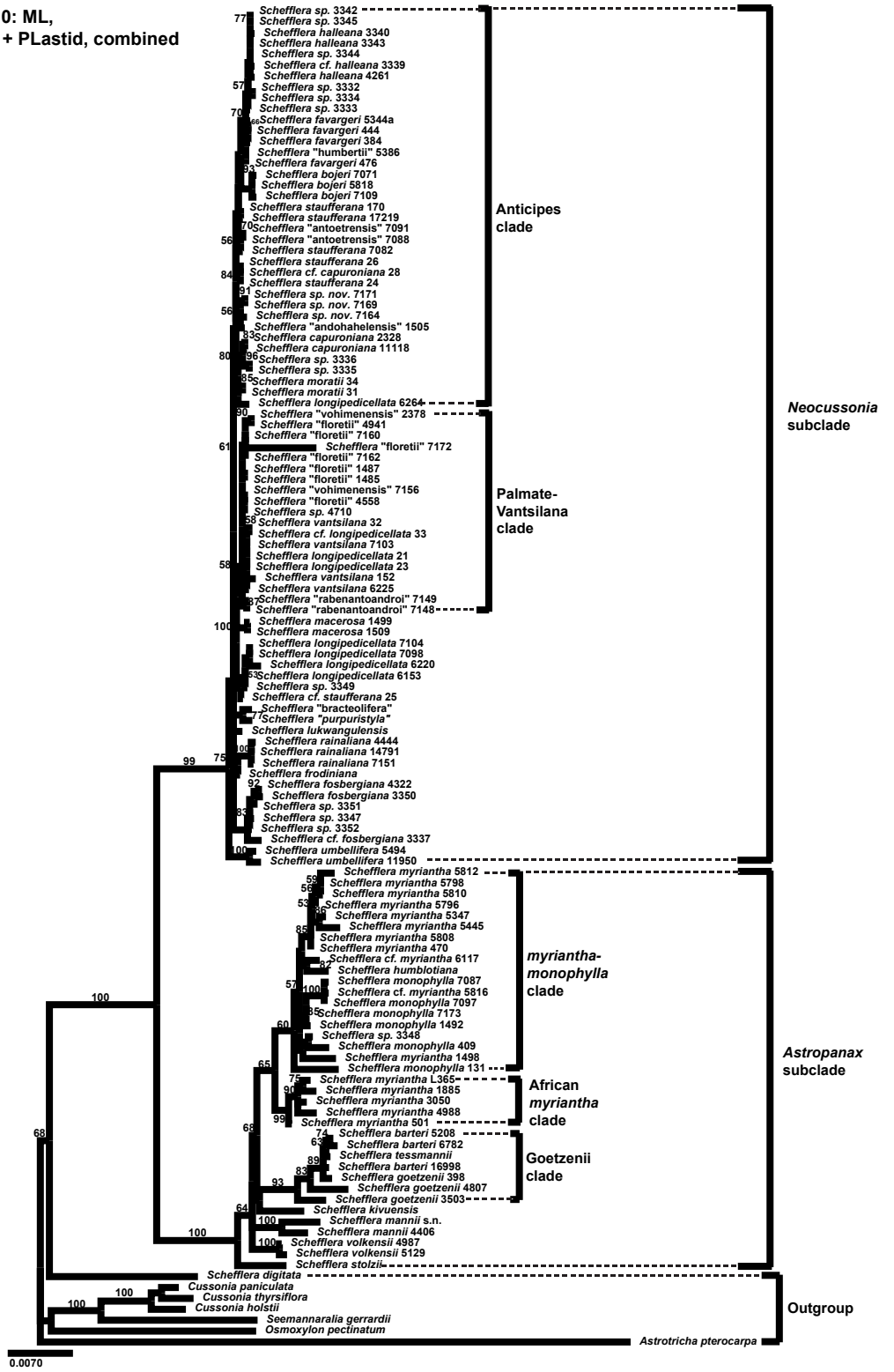


Figure 11: Bayesian, ETS

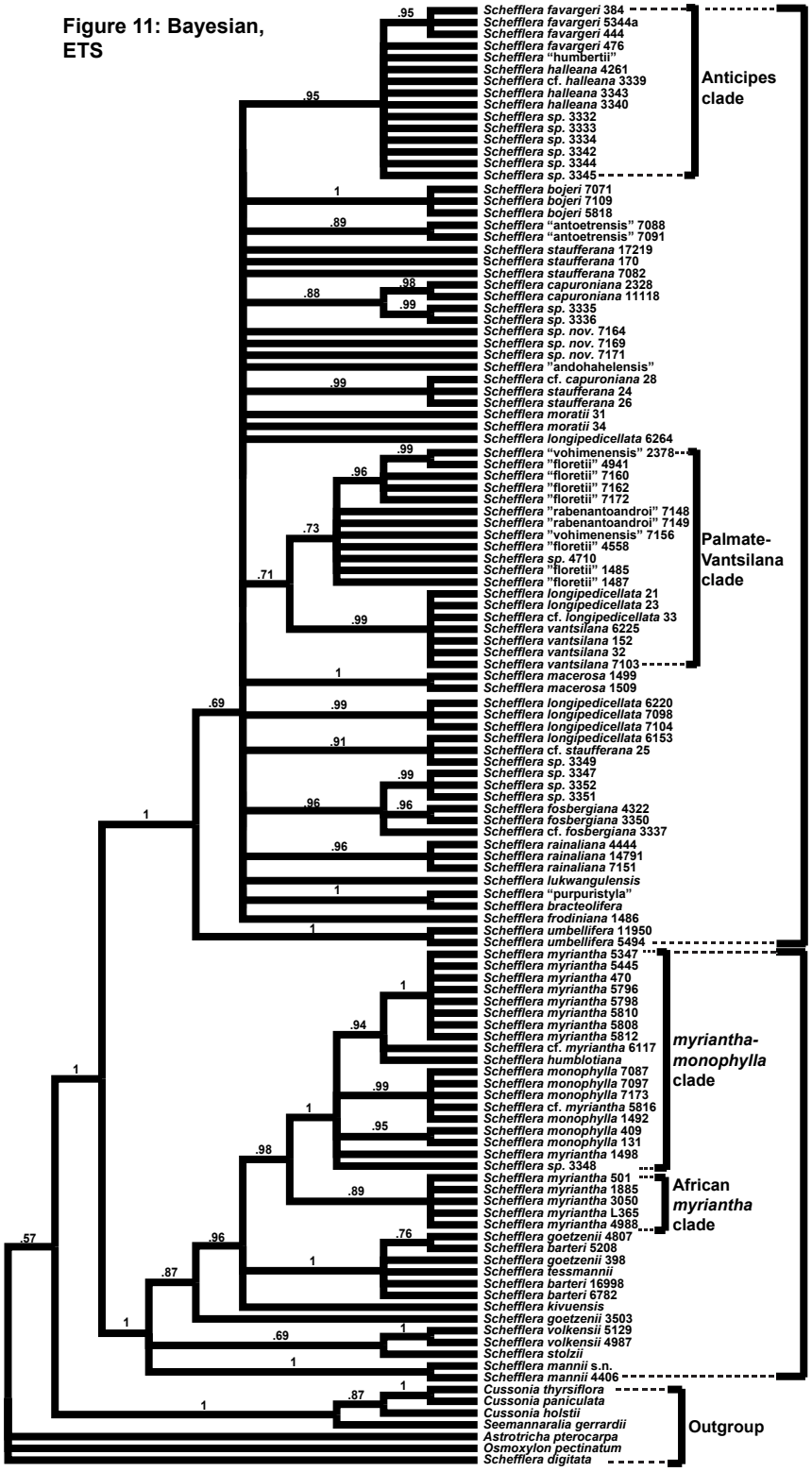


Figure 12: Bayesian, ITS

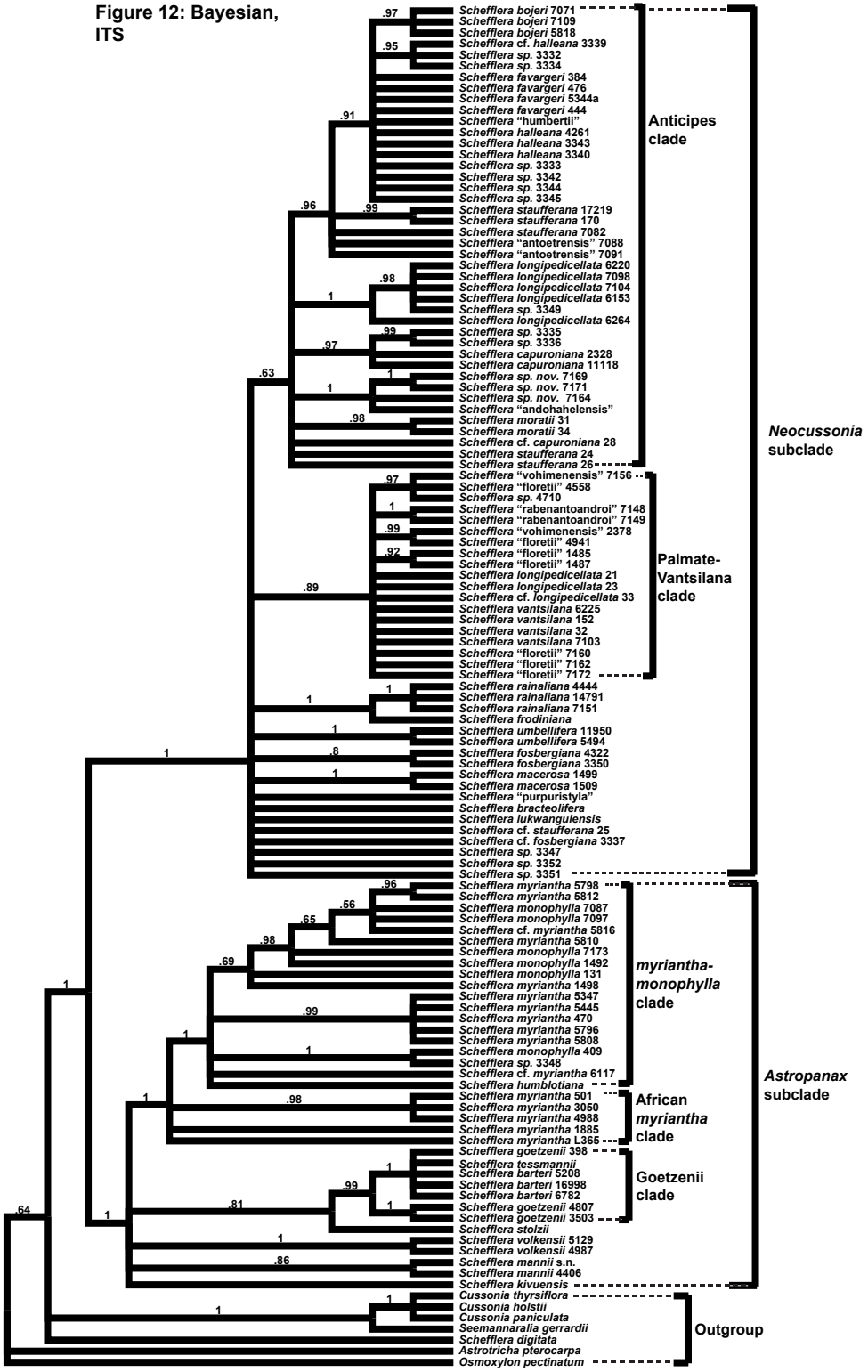


Figure 13: Bayesian, Nuclear, combined

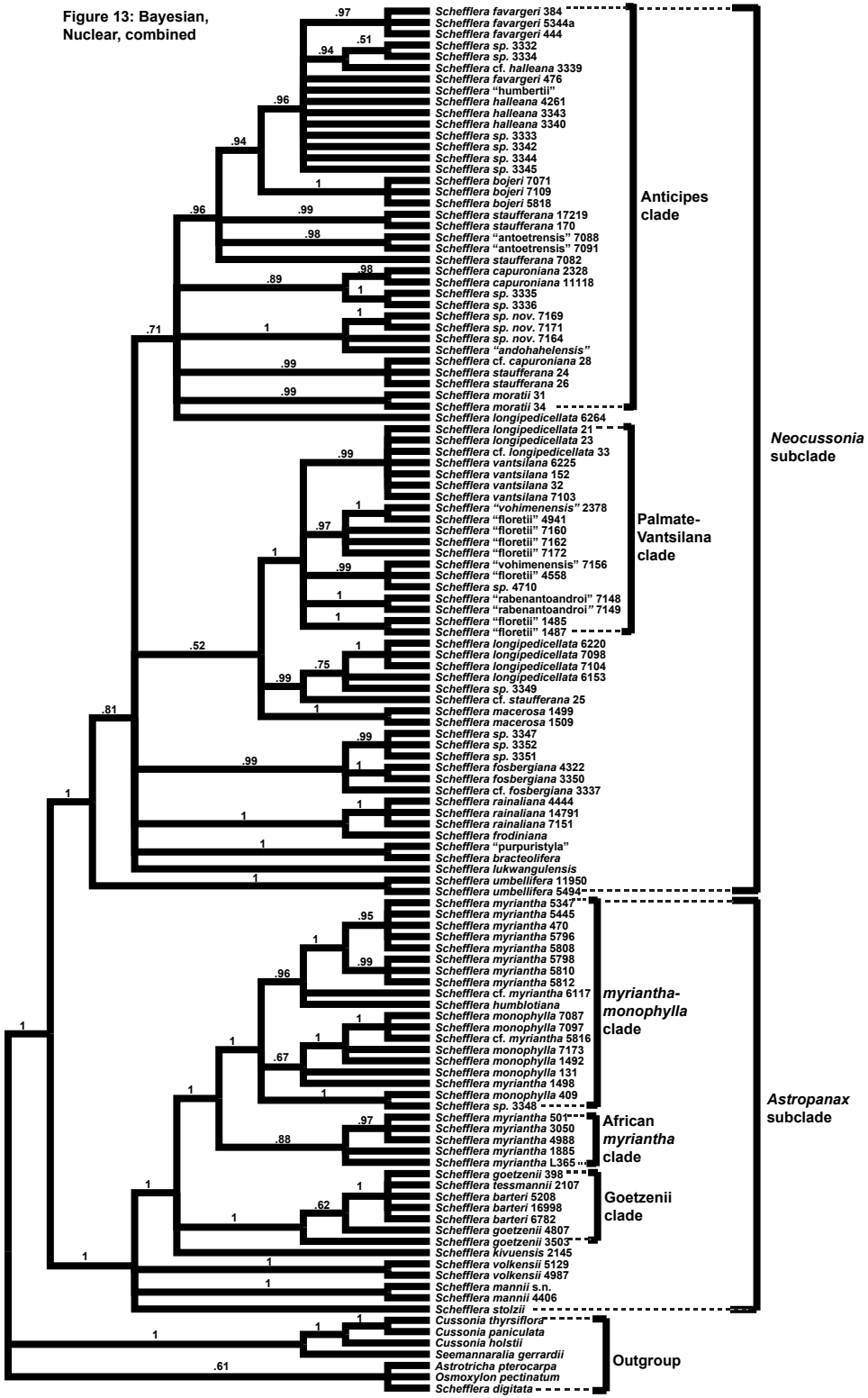


Figure 14: Bayesian, Plastid

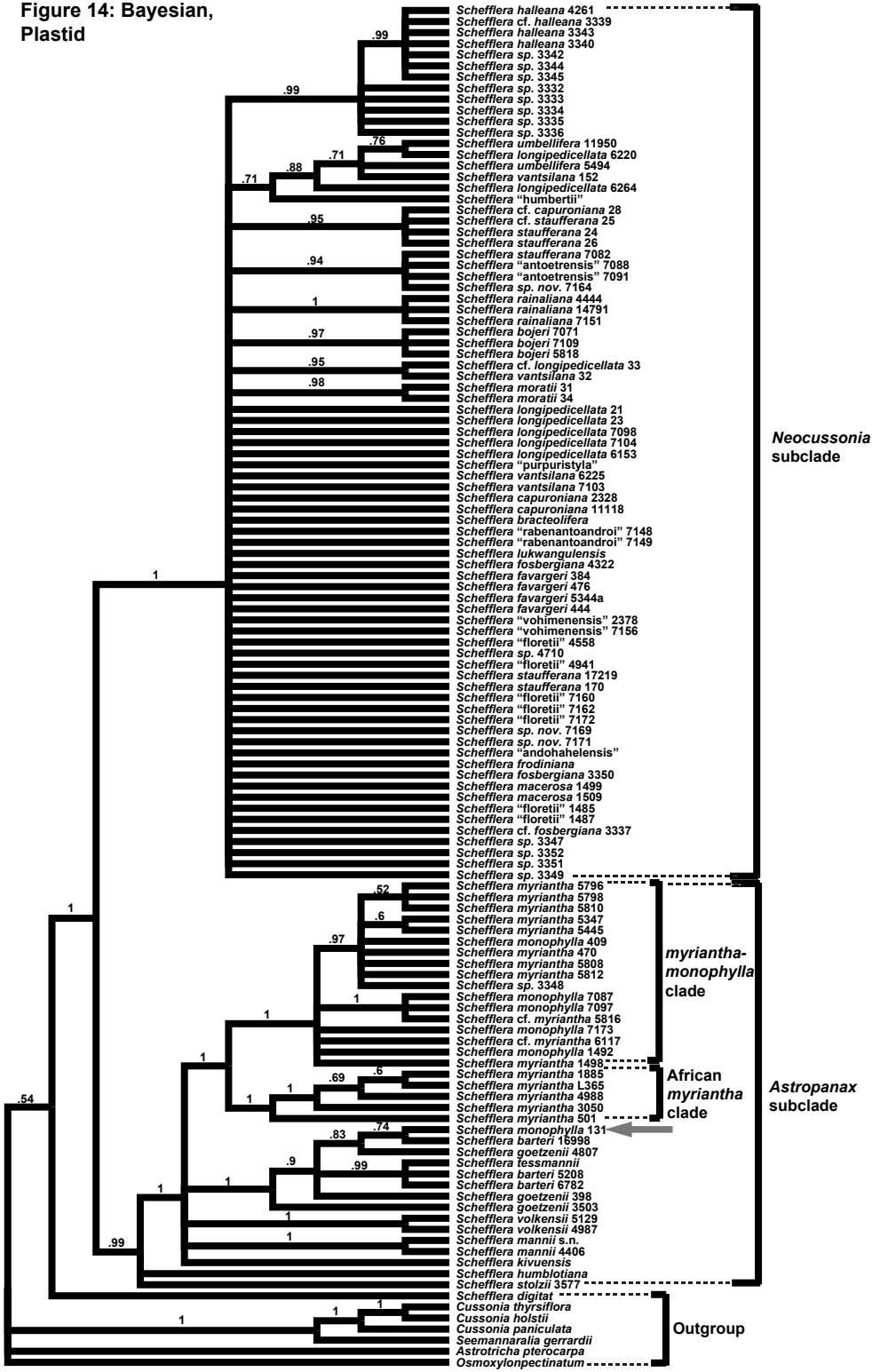


Figure 15: Bayesian, Nuclear + Plastid, combined

Figure 16: Geography

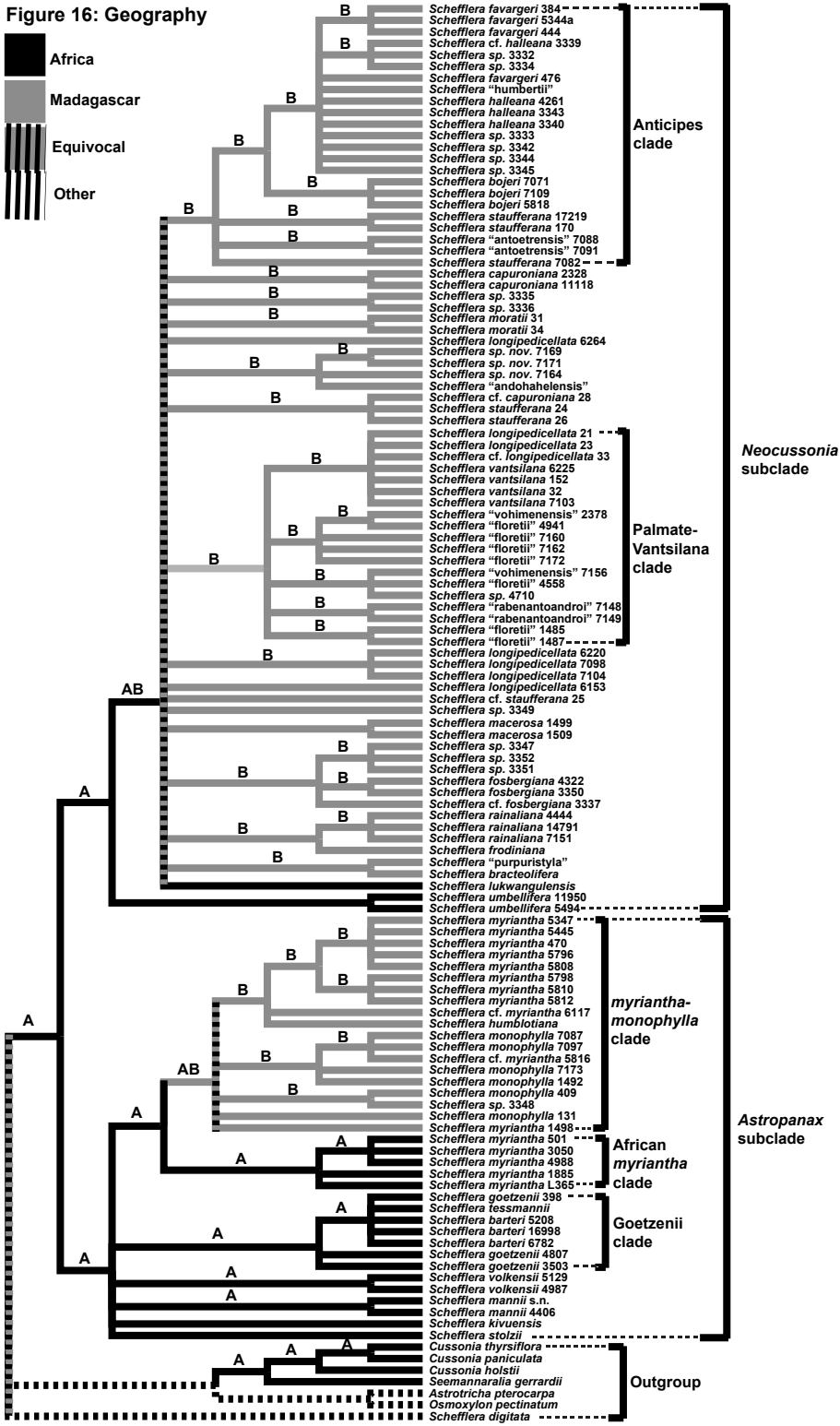


Figure 17: Carpel Number

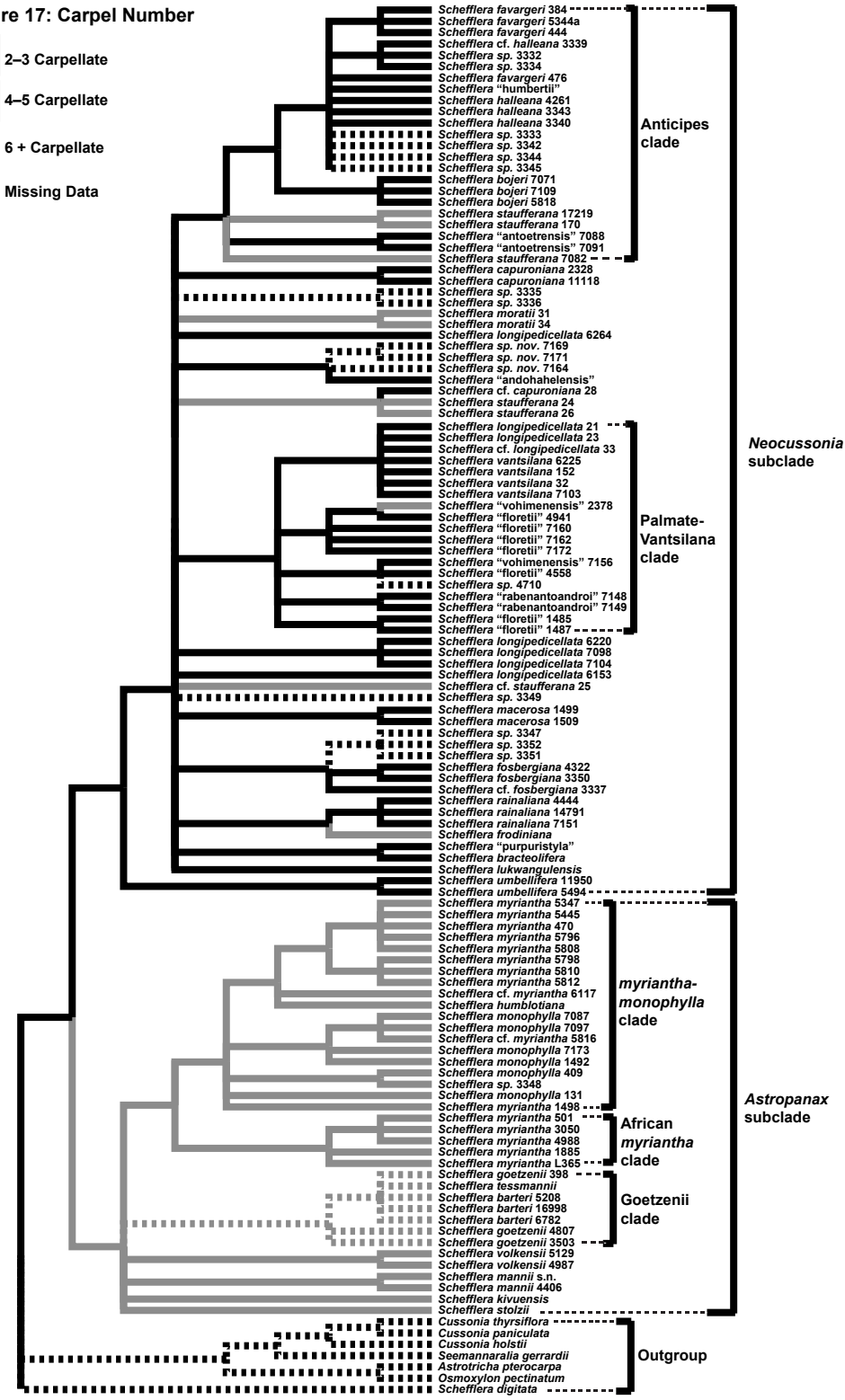


Figure 18: Leaf composition

Figure 19: Inflorescence Arrangement

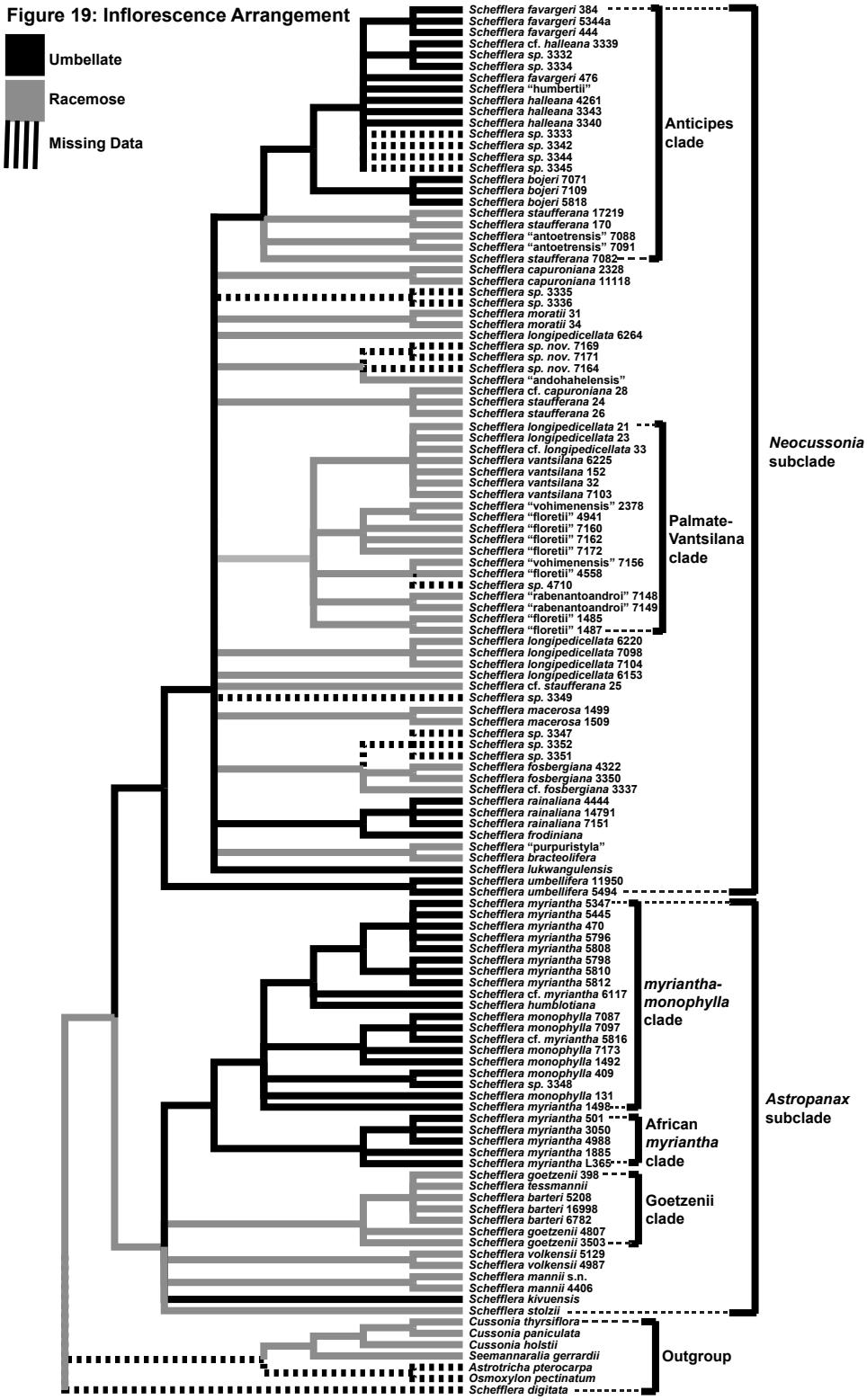


Figure 20: Pedicel length

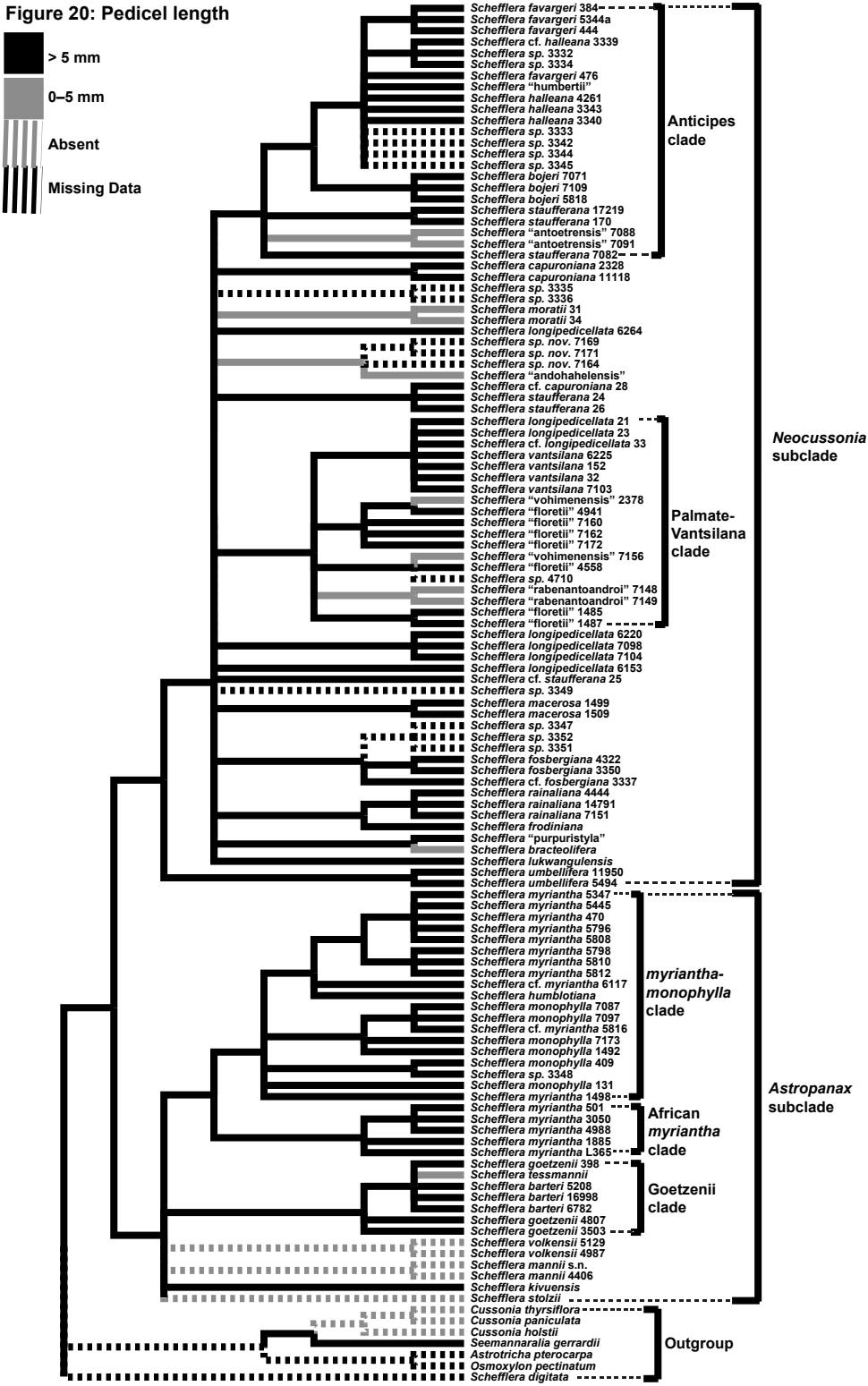
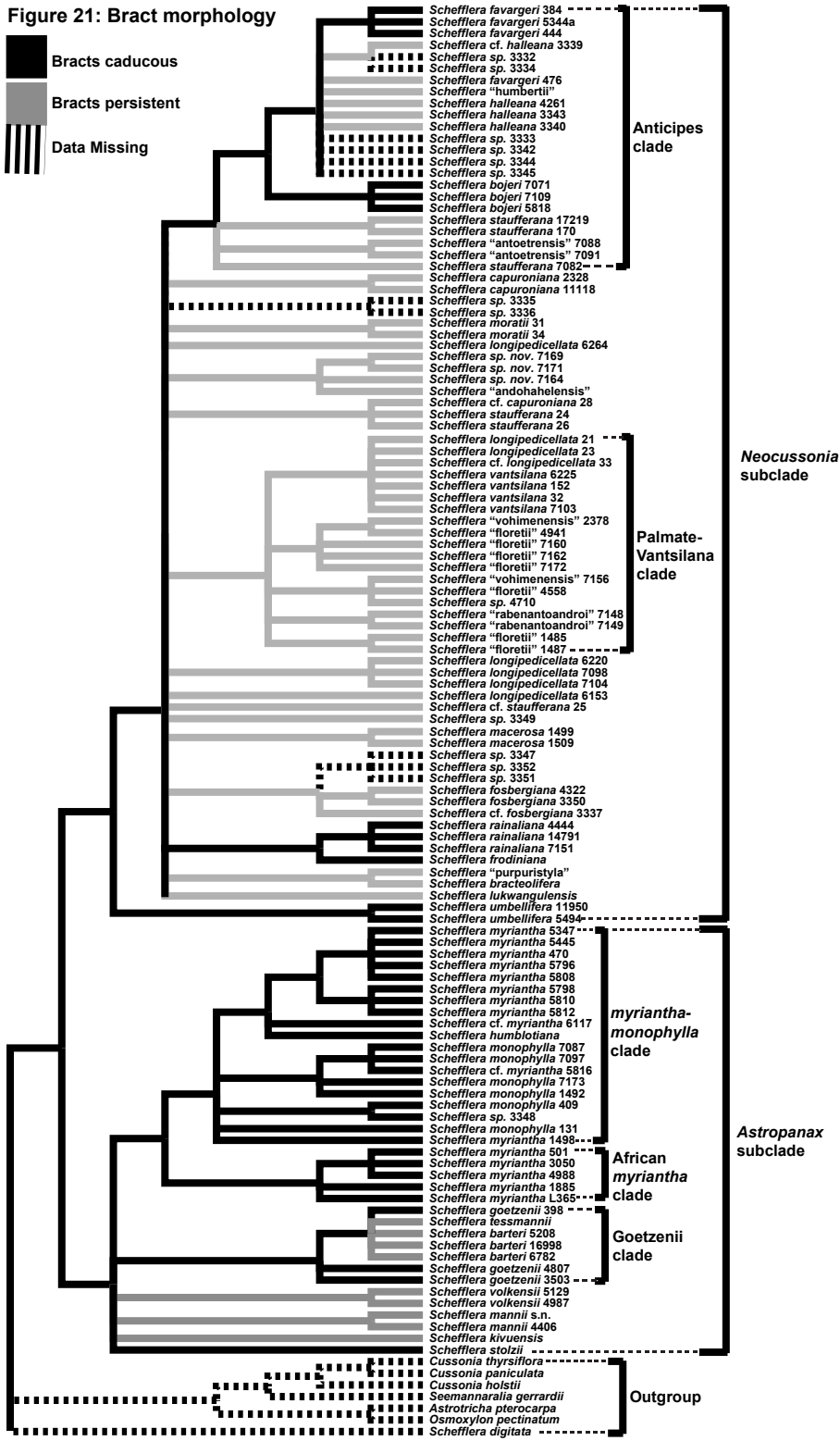


Figure 21: Bract morphology



Vita

Morgan Robert Gostel was born on January 30, 1985 in Henrico County, Virginia. He received his High School Diploma in 2003 from Douglas Southall Freeman High School in Henrico Country, Virginia. Morgan attended Virginia Commonwealth University as an undergraduate student and received his Bachelor of Science in Biology in 2008.